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Jean-Christophe Bambou

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Genetics and physiology of adaptation to gastrointestinal nematodes in small ruminants

Génétique et physiologie de l'adaptation des petits ruminants aux nématodes gastro-intestinaux

BAMBOU Jean-Christophe

Unité de Recherches Zootechniques,
Centre INRA Antilles Guyane, Domaine Duclos, 97170 Petit Bourg

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Rapporteurs :   Pr. Philippe Jacquiet, Ecole Nationale Vétérinaire de Toulouse
Dr. Dominique Martinez, Cirad Guadeloupe, HDR
Pr. Alessandro F. T. Amarante, Universidade Estadual Paulista, Brésil

Examinateurs :   Pr. Olivier Gros, Université des Antilles, Guadeloupe
Dr. Edwige Quillet, INRA Jouy-en-Josas, HDR

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A Geneviève, Salomé, Amandine et Rafaël

Foreword

Since my first steps in the world of research during my Bachelor of Science then my Master's Degrees at the Pasteur Institute[1] of Paris, I have been interested in the interactions between microorganisms and their hosts. We know that these interactions could be: i) beneficial for both the host and the microorganism (symbiotic mutualism), ii) beneficial only for one partner without deleterious effects on the other, (commensalism), iii) or deleterious for the host (pathogenic microorganisms). Initially, I worked on the interactions at the molecular level (characterization of surface receptors for entry into the cell of *Listeria monocytogenes*) and at the cellular level (*in vitro* and *in vivo* response of macrophages and gastric cells to *Helicobacter pylori* infection) during my Master's Degrees **(A1, A3, A8)**. Then, I addressed these interactions at the tissue level during my *PhD* thesis conducted in an INSERM[2] research unit (Hôpital Necker-Enfants Malades) (**A2, A3-A7**). Since 2005, when I arrived in the URZ (Unité de Recherches Zootechniques, Animal Production Unit), I have conducted research on host-parasite interactions at the whole organism scale.

This report is a synthesis of the work carried out since 2005 at the URZ and the PTEA facility (Plateforme Tropicale d'Expérimentation sur l'Animal) with the support of this rich ecosystem that is the couple URZ-PTEA in "symbiotic mutualism": administrative, technical and scientific staffs, and all our trainees, Academics, Engineers and Veterinarians. This work has been conducted in collaboration with colleagues from different fields: parasitologists (UMR IHAP[3] and Utrecht[4]), nutritionists (UMR SELMET[5] and URZ), molecular and quantitative genetics (URZ and INRA-GenPhyse[6]) and the PTEA facility.

I chose to present this work on two major themes: Improve the host response to gastrointestinal nematode (GIN) infection through the genetic way (Chapter III) and the nutritional way (Chapter IV). I chose to write the chapter III (my main topic during the last 10 years) as a literature review in which I highlight the findings on Creole goats rather than a discussion of the results. Probably influenced by my background in physiology, it appeared to me that it was important to describe the pathophysiology of these infections in ruminants (Chapter II). It was an essential prerequisite before addressing the characterization of the mechanisms of resistance and their interactions with the host nutritional status. An overall

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[1] http://www.pasteur.fr/en
[2] Institut national de la santé et de la recherche médicale (http://www.english.inserm.fr/)
[3] Interactions Hôtes Agents Pathogènes (INRA, Ecole Nationale Vétérinaire de Toulouse)
[4] Department of Infectious Diseases and Immunology, Netherlands, Molecular Biology and Parasitology
[5] Unité mixte de recherche Systèmes d'élevage méditerranéens et tropicaux, Montpellier
[6] Génétique, Physiologie et Systèmes d'Elevage, Toulouse

contextualization was also necessary to locate this topic over the global challenge of food safety and security in a changing world (Chapter I). References in this form (**letter number**) correspond to my work (**A**: ISI indexed publication; **B**: Manuscript submitted; **C**: Conference presentation; **D**: report diploma) (Appendix 1).

Chapter I: General introduction

1. Global context

According to the official United Nation population estimates and projection in 2012, the world population should reach 9.6 billion by 2050 (http://www.un.org). In developing countries, the population would increase by 70% to reach 8.2 billons, while the population in developed countries is expected to increase slightly from 1.25 billion in 2013 to 1.3 billion in 2050. This population growth and urbanization will fuel the increase in meat and milk consumption (Delgado, 2003; Herrero and Thornton, 2013). One way to meet this challenge of food security by 2050 is to rapidly improve efficiency in productivity and resources utilization in livestock farming systems with reduced environmental impact. In developing countries, small ruminants production is of interest for food security but not only, as it participates in the subsistence of a large human population by providing tangible (milk, meat, fiber, manure and cash) and intangible benefits (prestige, saving, insurance, cultural and religious purposes). As an example, in numerous studies based on field surveys, goats' meat stands out as the major source of animal proteins for many subsistence farmers (Dhanda et al., 2003). Indeed, goats are usually described as efficient converters of poor-quality feed into quality meat and milk, and for their ability to survive in some of the most inhospitable regions of the world (Norman, 1991). Thus, goat is usually called the 'poor man's cow' which underlines its importance in small farming systems. Recent reports from the Food and Agriculture Organization (http://www.fao.org) showed that in comparison to sheep, the goat population is expanding and more than 60% of this population is found in Asia and more than 95% in developing countries.

2. Gastrointestinal nematode parasitism in small ruminants production

Management of animal health is one of the corner stones of efficient livestock farming. In this context, gastrointestinal nematode (GIN) infections are one of the major pathogenic constraints on efficient grazing ruminants production system (Bishop, 2012; Charlier et al., 2014). In Australia, the United States and Argentina, estimates of economic losses have been done and range into tens of millions of dollars per year and concern all phases of production (Gibbs and Herd, 1986; McLeod, 1995; Fernández, 1997). The most susceptible physiological stages to GIN infections are young animals and periparturient does and ewes (Okon, 1980; Rahman and Collins, 1992; Barger, 1993; Chartier et al., 1998;

Schallig, 2000). The physiology of the immune system is implicated in both cases: in young animals there is an inability to develop effective acquired immune response due to immunological hypo-responsiveness and in periparturient does and ewes, a temporary relaxation of acquired immunity. A reduction of up to 60% of body weight gain has been reported in lambs (Coop et al., 1977; Abbott et al., 1986; Datta et al., 1999). In Creole does the GIN infection during lactation leads to lower weaning weight of kids and a 25% increase of the risk of death after weaning (Mandonnet et al., 2003; Mandonnet et al., 2005). It has been estimated that the presence of anthelmintic resistance resulted in a 14% reduction in carcass value in lambs and a reduction of goat farm profit of 81% (Sutherland et al., 2010; Gunia et al., 2013).

In temperate and tropical regions of the world the economically important GIN parasites of small ruminants belong to the order Strongylida and the family Trichostrongyloidae (Anderson, 2000). In cool temperate regions, the most economically important nematode parasite of small ruminants is *Teladorsagia circumcincta*, and in tropical and sub-tropical areas it is better *Haemonchus contortus* (Waller and Chandrawathani, 2005; O'Connor et al., 2006). However, in contrast with *T. circumcincta* an increasingly common occurrence of *H. contortus* have been reported also in temperate areas even up to latitudes above to 65.8N (Lindqvist et al., 2001; Hoste et al., 2002; Waller et al., 2004; van Dijk et al., 2008). Several decades ago, the biological plasticity of *H. contortus* to overcome unfavourable conditions either in the external or in the host environment (larval hypobiosis) has been reported in England (Connan, 1975; Waller and Thomas, 1975). Furthermore, according to the recent estimates from the Intergovernmental Panel on Climate Change (http://www.ipcc.ch), the temperature is projected to increase from 1.8 to 4.0°C from 1980-1999 to 2090-2099. Heat waves are very likely to occur more frequently and last longer, thus alleviating constraints on the development of GIN during the cold winter months of temperate countries. In this context, a simulation study revealed the potential for an increase in annual infection pressure of *H. contortus* and *T. circumcincta* in small ruminants (Rose et al., 2015).

Today worldwide there is an alarming rise of anthelmintic resistant GIN (Jackson and Coop, 2000; Papadopoulos, 2008). In addition, the use of anthelmintic is counter to the legitimate consumer concern about the presence chemical residues in animal products and the potential environmental consequences of anthelmintic (Beynon, 2012). It should be noted that in rural communities the use of anthelmintic is further complicated by a dearth of veterinary services and their high relative cost. Further, the issue of animal welfare also arises given the close relationship with animal health. Consequently, today a global scheme of parasitism

management integrating a parsimonious use of classical practices and alternative control strategies has to be developed. The objective is no more the total eradication of the parasite population in the flock but better the control of nematode populations in order to reach a favourable equilibrium for animal production. This integrated management aims to avoid parasite escape to the controls and decrease the risk of parasites evolution toward increased resistance to anthelmintics and virulence. To date, two main areas of research have been developed: firstly as short-term strategies, the reduction of host contact with infective larvae though different methods of grazing management, the management of nutrition to increase the capacity of the host to counter the deleterious effects of the parasite either by the development of a protective immune response (resistance), or by minimising the pathophysiological consequences of the infection (resilience), and the use of plant-derived bioactive substances (van Wyk and Bath, 2002; Hoste et al., 2008; Torres-Acosta and Hoste, 2008); secondly the improvement of the host response against GIN though the genetic selection of lines or breeds of resistant animals (Baker and Gray, 2003).

Part of the genetic variation between individual animals or breeds in the resistance against GIN is known to be under genetic control (Bishop and Stear, 2003; Gruner et al., 2004). The feasibility of different selection program has been studied worldwide, in both temperate and tropical conditions mainly in sheep and to a less extent in goat (Gray, 1997; Mandonnet et al., 2001; Vagenas et al., 2002; Fakae et al., 2004). Most of the time, selection is based on the phenotyping of relevant traits such as zootechnical performances, fecal egg count and measures of anaemia and blood eosinophilia under conditions of either nematode natural or experimental infection. Such breeding programs which requires adequate infrastructure to collect blood and faeces samples and perform the analysis during the course of GIN infection, are time consuming and costly. According to Bishop, it is unlikely that genetic markers (*i.e.* Quantitative Traits Loci, QTL) will make significant contribution to such breeding programs, in particular in developing countries essentially for practical reasons (Bishop, 2012). In contrast, the characterization of the underlying mechanisms should probably provide new biological markers (biomarkers profiles) predictive of the resistance and/or susceptibility to GIN infection phenotypes that could then improve the efficiency and timeliness of these breeding programs.

It is well established that the immune response against invading pathogens is costly in nutrients, and as a consequence sensitive to the host nutritional status. Thus, nutritional manipulation of small ruminants has long been considered as a tool for the control of GIN infections (Torres-Acosta et al., 2012). Further, by the use of a mathematical model, Vagenas

and colleagues showed a higher significant effect of the nutritional status on GIN resistance traits in sheep than the effect of the host genotype, suggesting that discrepancies between published genetic parameters for output traits, including faecal egg count (FEC), worm burden, growth rate and feed intake, may be function of environmental factors rather than differences in host genotype (Vagenas et al., 2007a; Vagenas et al., 2007b). Altogether these results suggest that the interaction host genotype × nutritional status should be considered in research works on the characterization of the determinism of genetic resistance.

Chapter II: Pathophysiology of GIN infection in small ruminants

1. Life cycle of GIN

The major species of gastrointestinal nematodes infecting grazing ruminants have a direct life cycle divided in two phases: the free-living phase in the external environment and the parasitic phase within the gastrointestinal tract of the host (Figure 1). Eggs are excreted with the feces of infected animals, then the pre-infective larval stages (L1 and L2) feed on microorganisms in the soil and infection is acquired by ingesting forage contaminated with the third stage larvae (L3). This external free living phase lasts 7 to 10 days under optimal conditions (high humidity and warm temperature). Infective larvae penetrate the gastric glands or the intestinal mucous membrane where they molt into L4. After 8-14 days they emerge and moult into L5 (pre-adult) before maturation into sexually active adults. Eggs excretion in the feces starts about 18-21 days after L3 ingestion (this is the prepatent period).
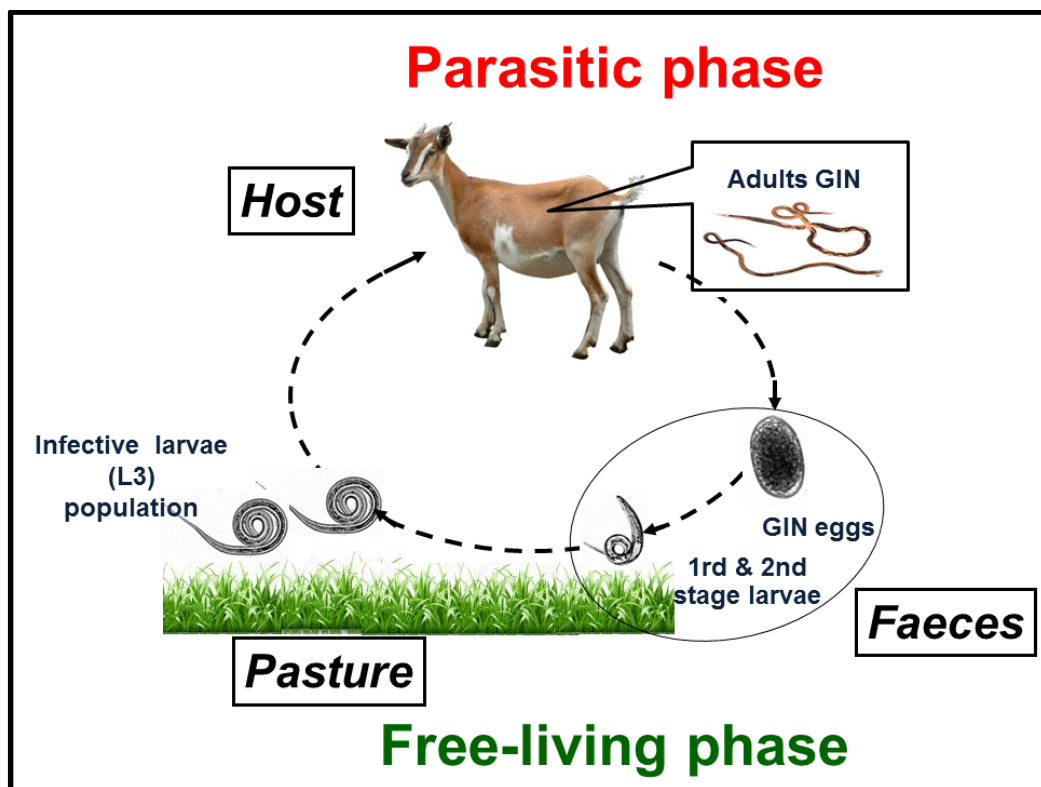

Figure 1. Schematic representation of gastrointestinal nematode life cycle

2. Impact on the gastrointestinal tract functions

The establishment of the GIN induces considerable mucosal injuries. The penetration of the abomasal gland or the mucous membrane by the larval stages induces a severe mucosal hyperplasia, nodules development and diffuses inflammatory cell infiltration. In the gastric glands, functional HCl secreting wall cells are replaced by undifferentiated cells. It has been shown in sheep infected with *T. circumcincta* that the number of functional cells can be halved after only 8 days of infection (Scott et al., 1998). Consequently the abomasal pH increase, which in turn induces hyperpepsinogenaemia, hypergastrinemia, reduces protein digestion, increases mucosal permeability and thus hypoproteinemia. Moreover, the raise pH allows the survival of rumen bacteria in the abomasum, which may reduce the availability of bacterial protein to the host (Nicholls et al., 1987). Major changes in abomasal secretions arise 2-6 days after L3 ingestion, at the time of larval emergence from gastric glands (Anderson et al., 1976; Anderson et al., 1981; Simpson et al., 1997). However, the adult stage of GIN is also implicated in the altered secretory function of the abomasum, since the transfer of adult *T. circumcincta* or *H. contortus* to recipient sheep (canulated sheep) induces an increase of the abomasal pH, serum pepsinogen and gastrin within a day (Anderson et al., 1985; Simpson et al., 1997; Scott et al., 1998). A rapid recovery of the capacity of the abomasum to acidify its contents has been observed after anthelmintic treatment which suppress the inhibitory effect of the parasites (Scott et al., 2000). Altogether these results suggested that the excretory/secretory products (ESP) of the parasite may inhibit the parietal cell secretory function. Indeed, it has been shown *in vivo* that the implantation of adult GIN confined in porous bags in sheep induced an increase of the abomasal pH (Simpson et al., 1999). Whether the inhibitory action of the ES products from GIN on the secretory function of the abomasum is direct or indirect remains unclear. However, this mechanism seems to be a key point of the infective process, since low pH is not optimal for GIN fecundity and survival (Honde and Bueno, 1982a, b; Simcock et al., 1999; Lawton et al., 2002).

The physiological pattern of gastrointestinal motility and digesta flow are also markedly disturbed by GIN infection. In the few experiments where it has been studied, a significant reduction of the motility and the passage of digesta have been shown is sheep infected with either *H. contortus* or *T. colubriformis* (Bueno et al., 1982a; Bueno et al., 1982b; Gregory et al., 1985). Such changes appears to be due both to the reduced feed intake, the increase circulating levels of gastrin secreted in response to the raise pH and the onset of diarrhea following the increase of the bacterial populations in the abomasum and the duodenum.

3. Impact at the organism level

3.1. Feed intake

Apart anemia induced by hematophagous parasites like *H. contortus*, the two major factors contributing to the reduced performance of parasitized ruminants are a reduced utilization of nutrients and voluntary feed intake (anorexia) (Kyriazakis et al., 1998). It is tempting to hypothesize that this is the result of the pathogenic effects on the gastric and the intestinal mucosa of GIN described above. A second theory which seems at first sight contradictory is that anorexia would be a behavior strategy that enables the host to avoid further infective larvae intake and to cope with the deleterious consequences of infection. Recently, Kyriazakis put forward the hypothesis that, anorexia can be viewed as both an unavoidable consequence of infection and a behavior strategy (Kyriazakis, 2014). Nevertheless, benefits linked to pathogen-induced anorexia would probably require fine homeostatic control, as chronic undernutrition has deleterious consequences for host defense.

The animal physiological status should probably be also considered since it has been suggested that anorexia is likely to be related to the acquisition phase of the immune response in young parasitized lambs (Greer et al., 2005). The interaction between the host genotypes differing in the level of resistance/susceptibility against GIN infection and the voluntary feed intake has also been addressed in few studies. No difference was observed in the voluntary feed intake between the line selected for low FEC (resistant) and the control unselected lines of Merino sheep when either infected or not with *T. colubriformis* and *T. circumcincta* (Liu et al., 2005; Doyle et al., 2011). However, discrepancies exist between the breeds since a transient small but significant depression of the voluntary feed intake was observed in Santa Ines lambs infected with *T. colubriformis* (Cardia et al., 2011). In Creole kids differing in the genetic resistance to GIN, we evaluated the effect of infection with *H. contortus* on voluntary feed intake and digestibility (**A11**). The experiment was carried out during two consecutive experimental infection of Creole kids initially naïves (primary and secondary infection). No reduction of feed intake was observed during the experimental infections. The diet digestibility decreased significantly but slightly (5% reduction) during the course of the infection but no difference was observed between the two genotypes. In a second study we addressed the question of the impact of the type of infection (trickle which better mimic the natural infection at pasture vs single) on the voluntary feed intake and the diet digestibility (**A16**). A significant transient reduction (10% reduction) in voluntary feed intake was observed only during the third week of the primary infection and no significant effect of the type of infection was observed. Altogether these results suggest that the slight (at the animal

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scale) negative effects of *H. contortus* infection on intake and digestibility are influenced both by the immunological stage and the level of resistance/susceptibility of the host genotype. However these negative effects could deeply impact the productivity at the flock, the territory scale.

3.2. Energy and protein metabolism

Losses of proteins in the gastrointestinal tract together with the reduction in dietary energy digestibility have been described in GIN infected ruminants. Increased level of plasma urea and higher excretion through urine have been observed in *T. circimcincta* and *H. contortus* infected sheep (Parkins et al., 1973; Roseby and Leng, 1974; Sykes and Coop, 1976; Roseby, 1977). The decrease in protein digestion and absorption and the loss of endogenous proteins are influenced by the parasite specie. The haematophagous parasites such as *H. contortus* induce mainly direct loss of protein through blood losses whereas others GIN appear to reduce reabsorption of endogenous protein (Poppi et al., 1986). A poor re-utilization of the absorbed nitrogen for *de novo* protein synthesis has been suggested since a higher level of non-urea nitrogen excretion was measured in *H. contortus* infected sheep (Rowe et al., 1988). Similarly in pair-fed studies, efficiency of metabolizable energy utilization for growth measured through the reduction of fat and protein deposition, was reduced by about 30 to 37% in sheep infected with either *T. colubriformis* or *T. circumcincta* (Sykes and Coop, 1976; Sykes et al., 1977; Coop et al., 1982). Similarly, we evaluated the interactions between GIN infection and diet supplementation on performance and carcass quality of growing Creole kids (**C21**). The liver and reticulorumen weights increased significantly in infected kids. A difference of 10.5 % of carcass yield was observed between infected non-supplemented and control non-infected kids. It has been suggested that the most important factor responsible for the reduced retention of digestible energy is an increase of the synthesis rates of protein in the liver and the gastrointestinal tissues (Jones and Symons, 1982).

In conclusion, we have seen in this chapter that GIN infection disturbs the nutritional status of their ruminant host by reducing feed intake, diet digestibility and absorption and nutrients metabolism and by increasing endogenous protein loss into the gastrointestinal tract.

Chapter III: Improve the host response through the genetic way
(from genetic variation to the underlying physiological mechanisms)

1. Genetic variation between and within sheep and goat breeds

Abundant knowledge has been accumulated on this topic since 80's. The early programs to examine and to understand the mechanisms underlying the genetics of resistance in sheep were initiated in Australia (Woolaston and Gray, 1991) and New Zealand (Watson et al., 1986), (amongst the largest sheep producing countries of the world), as they were intensively exposed to parasitism and anthelmintic resistances. In tropics, more precisely in developing countries of the tropics, the less pronounced intensification (multi-purpose breeding, lower artificialisation of the environment) together with the maintenance of indigenous breeds adapted to their environment has allowed the preservation of genotypes more resistant to GIN infections. In sheep, a better capacity of local breeds native from humid areas compared with the more commercial ones, to express a resistant/resilient phenotype has been shown (lower FEC, parasite burden and packed cell volume reduction). Local breeds from South America, the Caribbean and Asia, such as the Santa Ines, Crioula lanada, Criollo, Blackbelly, Florida native and Garole breeds, at different physiological stages (*i.e.* growing lambs, adult male and female around parturition) showed a higher level of resistance against GIN compared with Ile de France, Corriedale, Suffolk, Romane, Rambouillet and Decanni breeds respectively (Courtney et al., 1984; Bricarello et al., 2002; Nimbkar et al., 2003; Amarante et al., 2004; Bricarello et al., 2004; Alba-Hurtado et al., 2010). In goats, a few number of studies compared different breeds in tropics for this trait (De la Chevrotiere et al., 2011). It has been postulated that specialized breeds are not able to express their genetic potential of production under harsh environment, due to their higher nutritional requirements (Hoste et al., 2001).

Genetic variation for resistance to GIN has also been investigated within breeds. In sheep, Safari et al (2006) calculated the weighted mean of FEC heritability estimates in the literature at 0.27. In goats, heritability of resistance to GIN is about half of that calculated in sheep (Costa et al., 2000; Bakert et al., 2001; Mandonnet et al., 2001; Chiejina and Behnke, 2011; Rout et al., 2011). Interestingly, the genetic control of GIN infection in both sheep and goats appears to be non-specific to the nematode species, at least partially (Gruner et al., 2004) **C13**). Furthermore, genetic correlations between FEC and body weight vary from

favorable negative values to unfavorable positive values (Bakert et al., 2001; Safari et al., 2005; Gunia et al., 2011). This variation may be due to interactions between host genetic resistance and the environment (Laurenson et al., 2012). In Creole kids, increasing genetic variability was assessed between 3 and 11 months of age with decreasing maternal genetic effects with age (Mandonnet et al., 2001). Positive genetic correlation was estimated between resistance of growing kids and periparturient rise of does (Mandonnet et al., 2006). Otherwise, neutral relationship were shown between fertility, litter size, milking value and FEC while genetic correlation was slightly favorable between body weight and FEC (Gunia et al., 2011).

Many Quantitative trait loci (QTL) associated with resistance to GIN in different breeds of sheep have been detected on more than 20 chromosomal regions, as reviewed by Dominik (2005) and Bishop and Morris (2007). The first genome scan in goat was undertaken in Creole breed (**A15**) identifying 13 QTL for resistance, resilience and immune criteria. The main conclusion of these studies is that most significant QTL effects tend to be scattered throughout the genome. According to Bishop (Bishop, 2012), it is likely that resistance to GIN in small ruminants is probably driven by numerous genes with small effects and few playing a key role. This genomic information accumulates but remains difficult to exploit by professionals.

2. The mechanisms underlying the genetic resistance
2.1. Host immunity to GIN infection: the keystone of the genetic resistance?

Like most economically important infectious diseases in animal production, the immune response against GIN has been investigated since the early 70's mainly in sheep. It has been shown that the response against gastrointestinal nematodes is associated with proliferation of mucosal mast cells, globule leukocytes, and circulating and tissue eosinophils (O'Sullivan and Donald, 1973; Adams and Cripps, 1977; Miller, 1986). This response also involves production of parasite-specific immunoglobulin A (IgA), IgG1 and IgE (Cripps and Steel, 1978; Adams et al., 1980; Charleypoulain et al., 1984; Zajac et al., 1990; Cuquerella et al., 1991). In 1988, it has been suggested for the first time in outbred animals that the immune response was closely linked to the high level of resistance to GIN. Immune suppression with dexamethasone (a synthetic glucocorticoid with anti-inflammatory and immunosuppressive properties) abrogated the resistance to *H. contortus* of the genetically selected resistant line of

Merino sheep (Presson et al., 1988). A short time later, in that same line of Merino sheep, a strong evidence for a close association between the genetic resistance to GIN and the immune response was shown. After depletion of the CD4+ T helper (Th) cells abrogation of the resistance to *H. contortus* was again observed (Gill et al., 1993b).

More recently, studies have been conducted to identify the genes and the genes networks underlying the genetic resistance to GIN in sheep. By comparing the duodenal mucosa transcriptome of resistant and susceptible Perendale lambs either field infected or naïves, it has been shown that the differentially expressed genes were predominantly related to the development of acquire immunity and smooth muscle structure (Diez-Tascon et al., 2005; Keane et al., 2006). In infected lambs and in the naïves ones, the more highly expressed genes in resistant animals were implicated in the maintenance of a healthy immune system and in susceptible animals it was better genes related to cellular stress responses. The kinetic of the mucosal transcriptome was investigated in *H. contortus* and *T. colubriformis* infected resistant and susceptible sheep. In these studies the major gene changes were related to innate and adaptive immunity and mucosal maintenance (Ingham et al., 2008; Rowe et al., 2008, 2009). Altogether these studies strongly suggest that the genetic resistance to GIN in small ruminants is closely linked to the host immune response. However, even if the genetic control seems non-specific to the nematode species, it appears that the underlying mechanisms are different at least partly, from breed to breed (within sheep), between goats and sheep and depending on the parasite specie.

2.2. The protective immune response: the quantity or the quality, what really matters?

2.2.1. The humoral response

In sheep, numerous studies have been conducted with the aim to characterize the protective immune response associated with the genetic resistance. The strategy was generally to compare resistant and susceptible genotypes either within breed or between breeds. Thus, it has been shown that a large part of the protective immune response to GIN is mediated by the humoral response, especially by IgA and IgE. The IgA response has been described as the major effector mechanism that control nematode egg production. Indeed, a review that date from 1999 concluded that the IgA mediated suppression of GIN growth and fecundity was the major mechanism of resistance to *T. circumcincta* in lambs (Stear et al., 1999). The IgA response is also directed against the fourth-stage larvae (L4) since an increased number of inhibited L4 larvae together with a decrease number of adult *T. circumcincta* was found to be

associated with increased plasma IgA activity (Stear et al., 1995; Stear et al., 2004; Stear et al., 2009). A similar role of the IgA response in the control of *H. contortus* and *T. colubriformis* has also been suggested (McClure et al., 1992; Gill et al., 1993a; Douch et al., 1994; Strain and Stear, 2001) but the question of the association with the genetic resistance has been addressed mainly in *H. contortus* infected sheep. A high heritability ($h^2 = 0.57$) of the plasma IgA activity to L4 antigens was estimated in sheep, underlining the genetic basis of this effector mechanism (Strain et al., 2002; Davies et al., 2005). Thus, the level of IgA against the CarLA antigen (a carbohydrate larval surface antigen expressed on the L3 of all trichostrongylid nematode species (Harrison et al., 2003a; Harrison et al., 2003b) has been proposed to be a suitable means to measure the level of protection to GIN. Indeed, the CarLA saliva IgA antibody test is currently proposed as an accurate and simple (compared with FEC) way to undertake selective breeding (http://www.carlasalivatest.co.nz/Home.aspx). However, it should be noted that this tendency for a close association between the IgA activity and the genetic resistance has not been observed for all the resistant genotypes. Indeed, similar mean values of mucus IgA were found in the resistant Santa Ines and in the susceptible INRA401, and the susceptible Suffolk and Ile de France breeds of sheep (Amarante et al., 2005; Lacroux et al., 2006).

In the same manner, higher level of IgE against GIN antigens correlated with lower FEC were found in resistant compared with susceptible genotype of sheep (Huntley et al., 2001; Shakya et al., 2009; MacKinnon et al., 2010; Murphy et al., 2010). The underlying mechanism is probably a type 1 hypersensitivity reaction mediated by the proliferation of mucosal mast cell and the degranulation of the IgE-primed mast cells (Miller, 1996). The typical effectors of this reaction have been observed: increased mucus production (Stear et al., 2003), increased expression of interlectin (French et al., 2008) and increase mucosal infiltration of globules leukocytes (Huntley et al., 1992).

In goats, apart from our work on the Creole goats, this issue has been poorly investigated. Evidence for a humoral response (IgA and IgE) under partial genetic control in Creole goats has been shown (**A14**). High positive genetic correlations were found between the IgA response and FEC (0.84 and 0.72 for IgA anti-ESP (Excretory/secretory products) and IgA anti-L3 (L3 crude extracts) respectively, Table 1). Moreover, a moderate heritability of the serum IgA was found in this goat breed ($h^2 = 0.19$ and 0.23 for IgA anti-ESP and IgA anti-L3 respectively, Table 1) and a moderate genetic correlation (-0.32) was found between FEC and the IgE anti-L3 response ($h^2 = 0.44$). We concluded that the immune response involving activity of the IgE anti-L3 response may be a key component for the expression of

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the resistance phenotype in Creole goats. However, in contrast with the ovine studies a positive phenotypic correlation ($r = 0.59$) was found between the IgE response directed against adult *H. contortus* and the FEC in Creole goats, suggesting an hypersensitivity reaction dependent on worm prolificacy (**A9**).

Table 1. Phenotypic and genetic correlations between resistance, production traits and IgE response (A) and IgA response (B)

A

	FEC[1]	EOS[2]	PCV[3]	BW[4]	IgA anti-ESP[7]	IgA anti-L3[8]
FEC	0.20 (0.03)	-0.22 (0.08)	-0.35 (0.08)	0.0005 (0.08)	0.84 (0.13)	0.72 (0.18)
EOS	-0.11	0.20 (0.04)	-0.04 (0.10)	-0.07 (0.12)	-0.005 (0.31)	-0.44 (0.25)
PCV	-0.24	0.03	0.22 (0.03)	0.39 (0.08)	-0.36 (0.18)	0.04 (0.17)
BW	-0.10	0.04	0.49	0.32 (0.04)	-0.50 (0.15)	-0.38 (0.16)
IgA-ESP	-0.06	0.10	0.03	-0.02	0.19 (0.09)	0.83 (0.11)
IgA-L3	-0.04	0.10	0.004	-0.02	0.82	0.23 (0.11)

B

	FEC[1]	EOS[2]	PCV[3]	BW[4]	IgE anti-ESP[5]	IgE anti-L3[6]
FEC	0.21 (0.03)	-0.23 (0.06)	-0.35 (0.08)	-0.007 (0.06)	-0.08 (0.12)	-0.32 (0.08)
EOS	-0.11	0.20 (0.03)	-0.05 (0.07)	-0.09 (0.07)	-0.18 (0.14)	0.03 (0.09)
PCV	-0.24	0.03	0.22 (0.03)	0.40 (0.07)	0.18 (0.12)	-0.09 (0.06)
BW	-0.10	0.04	0.49	0.31 (0.03)	0.13 (0.21)	0.35 (0.16)
IgE-ESP	-0.14	-0.06	0.13	0.08	0.18 (0.10)	0.79 (0.11)
IgE-L3	-0.08	-0.03	-0.01	0.07	0.60	0.44 (0.13)

Phenotypic correlations are presented below the diagonal, heritability estimates on diagonal and genetic correlations above the diagonal
Standard deviations are shown between brackets

[1]FEC, Faecal Egg Count
[2]EOS, circulating Eosinophilia
[3]PCV, Packed Cell Volume
[4]BW, Body Weight
[5]IgE anti-ESP, IgE against Excretion-Secretion Products
[6] IgE anti-L3, IgE against *H. contortus* infective larvae (L3) crude extract
[7]IgA anti-ESP, IgA directed against Excretion-Secretion Products
[8] IgA anti-L3, IgA directed against *H. contortus* infective larvae (L3) crude extract

When we compare the response of immune mature Creole goats experimentally infected with *H. contortus*, despite a high difference in FEC between resistant and susceptible

animals (11 times higher in susceptible) no difference was observed in the levels of serum IgA and IgE directed either against L3 or adult antigens. We concluded that in immune goats a degree of protection occurred and the phenotypic and genetic segregation in resistant and susceptible animals were not related to the humoral immune response. Unfortunately, there is no data in the literature addressing the role of the humoral (IgA, IgE) response in the protective response associated with the genetic resistance in goats. Consequently, the discrepancy between our results in the Creole goat breed and the different sheep breeds could not be discussed as a difference between goats and sheep. The comparison of the Creole goat response with other goat breeds would be of great interest for the understanding of the mechanisms which could be specific to this species.

2.2.2. The cellular response

A cellular immune response mainly characterized by blood and mucosal eosinophila, mucosal mast cell hyperplasia and increased intraepithelial mucosal mast cell infiltration (the globule leukocytes) and the clonal expansion Th2 cells, a distinct lineage of CD4+ effector cells that secretes mainly IL-4, IL-5 and IL-13 (Figure 2). The latter remains under discussion since this immunological paradigm relating to the development of a Th1/Th2 cell dichotomy in ruminants is not fully demonstrated due in part to the lack of specific tools and reagents (Hope et al., 2012).
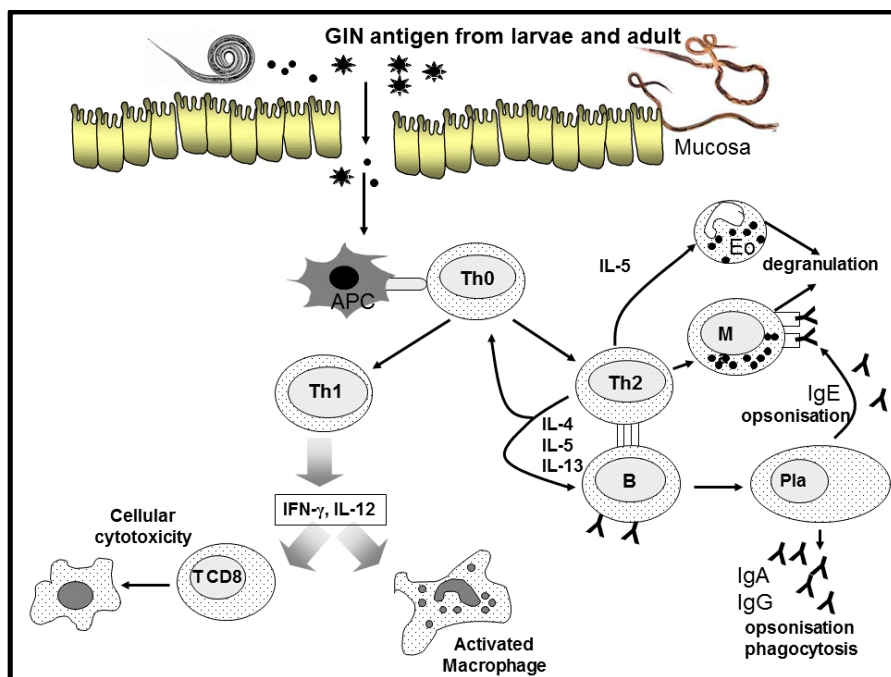

Figure 2. Schematic representation of the Th1/Th2 balance. GIN (Gastrointestinal Nematode), Th (Lymphocyte T helper), Eo (Eosinophil), M (Mastocyte), Pla (Plasmocyte)

It is generally assumed that blood eosinophils and the infiltration of target tissues by eosinophils are characteristic outcomes of helminth infection in mammals. Many studies suggested that this cell population plays a role in resistance to helminth infection since significant correlations between resistance/susceptibility to endoparasites infection and the magnitude of the peripheral eosinophil response has been shown (Meeusen, Balic, and Bowles, 2005). More recently it has been shown that eosinophils could interact with and damage gastrointestinal nematode larvae *in vivo* (Balic et al., 2006; Robinson et al., 2010) (Figure 3). Their potential to impair larval infectivity by *in vitro* pre-exposure has also been shown (Terefe et al., 2007a), Figure 4). However, this relationship is not observed in all studies, since it has not been found between tissue and circulating eosinophils and the adult parasite burden and FEC in *T. circumcincta* infected sheep (Henderson and Stear, 2006; Beraldi et al., 2008). It has been suggested that this ambiguity would be related to the fact that the direct contact between eosinophils and parasites is deeply reduced in *T. circumcincta* infection which causes little mucosal damage compared with *H. contortus* (Venturina et al., 2013).
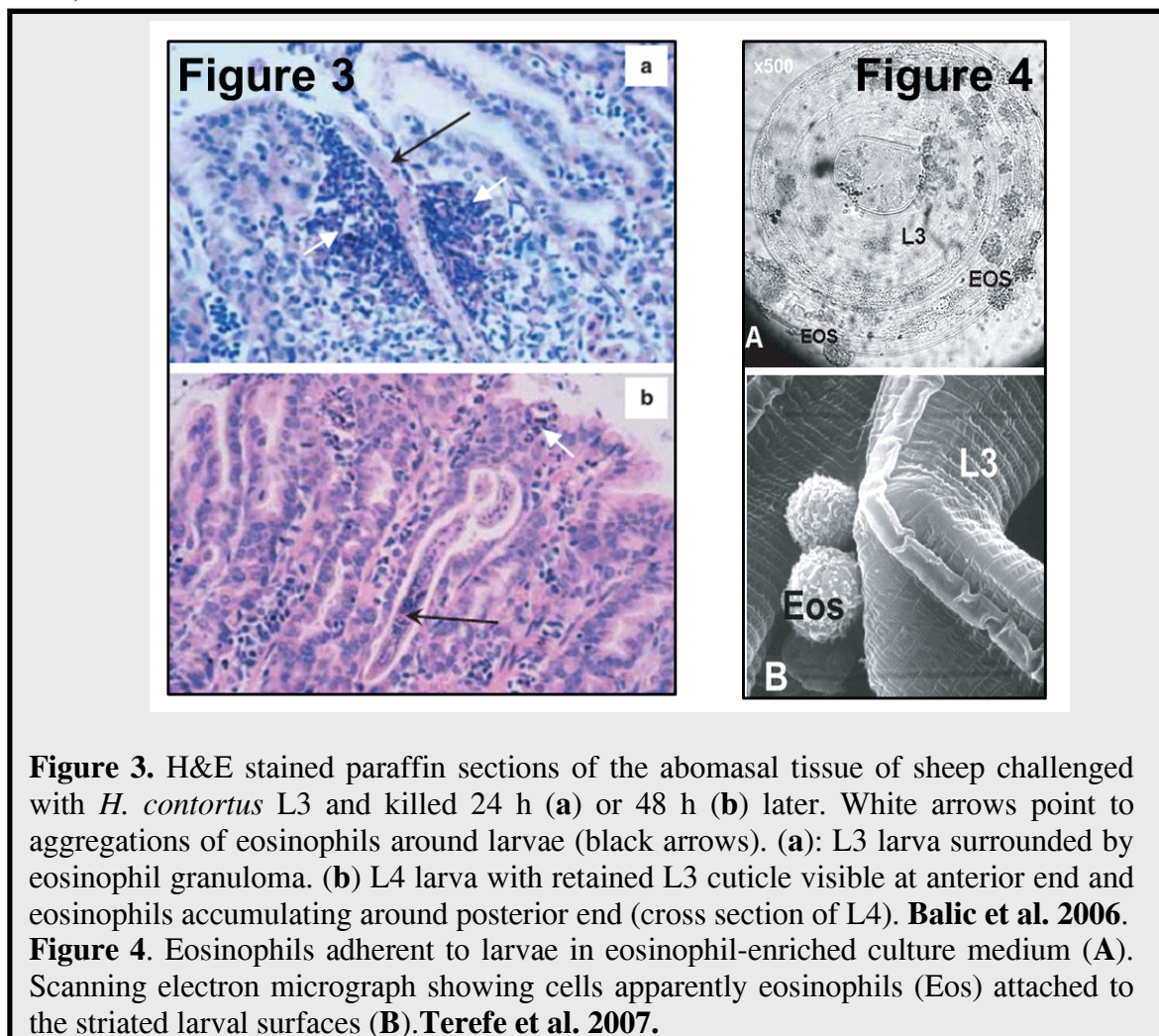
Figure 3. H&E stained paraffin sections of the abomasal tissue of sheep challenged with *H. contortus* L3 and killed 24 h (**a**) or 48 h (**b**) later. White arrows point to aggregations of eosinophils around larvae (black arrows). (**a**): L3 larva surrounded by eosinophil granuloma. (**b**) L4 larva with retained L3 cuticle visible at anterior end and eosinophils accumulating around posterior end (cross section of L4). **Balic et al. 2006**.
Figure 4. Eosinophils adherent to larvae in eosinophil-enriched culture medium (**A**). Scanning electron micrograph showing cells apparently eosinophils (Eos) attached to the striated larval surfaces (**B**).**Terefe et al. 2007.**

In our Creole goat model, an increased blood eosinophils counts was observed after an experimental *H. contortus* infection, but no correlations between peripheral blood eosinophilia and protection/resistance were found (**A9**, **A10**, **A17**). We have even found a higher blood eosinophilia in susceptible compared with resistant kids experimentally infected with *H. contortus* (**A10, A17**). At first sight, it could be suggested that the role of the blood eosinophilic response would be different in goats compared to sheep. However, our data showed that blood eosinophilia at slaughter was negatively correlated with adult worm counts and with female worm length (**A17**). In this study the histological examination of the abomasal mucosa showed a more pronounced cellular immune infiltration after the primary infection than after the secondary infection and the intensity of the cellular infiltration was not affected by the genetic status, except for globule leukocyte infiltration which was higher in resistant animals after the primary infection (Figure 5). Moreover, medium negative phenotypic correlations were observed between globules leukocytes and immature worm burden whatever the genetic status. Previous studies in immunised sheep showed that globule leukocytes were implicated in the immune exclusion of challenge larvae (Huntley et al., 1992; Balic et al., 2002; Kemp et al., 2009). Recently, Robinson *et al* monitored the local cellular immune response in immunized sheep challenged with *H. contortus* and observed a peak of globule leukocyte infiltration in the abomasal mucosa at five days post-infection (Robinson et al., 2010). In accordance with this study, our data suggest that globule leukocytes would be implicated in the response against larval stage of *H. contortus* a mechanism associated with the genetic resistance. In contrast, previous studies in goats showed an abundant globule leukocyte infiltration in the abomasal mucosa over 10 weeks post-infection, suggesting that these cells would be associated with adult nematodes rejection (Perez et al., 2001). Interestingly, the non-specific CD79 (marker for B cell) immunoreactivity of globules leukocytes observed in our study (**A17**), suggested the heterogeneity of this cell population. To our knowledge this result has never been reported in the literature, but to date, the origin and the function of this cell population remain controversial (Spoor et al., 2011). Moreover, in Creole kids previously infected by *H. contortus* a degree of protection occurred and the phenotypic and genetic segregation in resistant and susceptible animals were not related to the circulating activated sub-populations of LTCD8+ and LTCD4+ (**A10**). It has been shown that a degree of protection occurred also in sheep exposed to repeated infection (Gregg et al., 1978; Gamble and Zajac, 1992). Recently, we showed that Creole kids exposed to high level of infection during the post-weaning period were also more resistant to a challenge *H. contortus* infection (**A19**). However, the question of whether the expression of the resistant

genotype was observed since the primary exposure to GIN infection has not been directly addressed in the literature. As an example, St. Croix lambs (resistant) developed significantly greater level of resistance to *H. contortus*, only after a primary exposure, as compared with Dorset lambs (susceptible) (Gamble and Zajac, 1992). In keeping, Terefe et al showed a significant reduction of the parasitological parameters in INRA 401 lambs (susceptible) after a primary infection (Terefe et al., 2007b). In contrast, resistance was expressed since the primary infection in Barbados Black Belly lambs (resistant). These results raise the question of the regulation of the protective immune response associated with the genetic resistance.

Figure 5. Globules leukocytes identification under light microscopy in the abomasal mucosa of kids experimentally infected with *H. contortus* (examples indicated with arrowhead). (**a**) Hemalun eosin saffron staining. Globoid cells are rounded, with a round nucleus and abundant cytoplasm filled with eosinophilic material and optically clear vacuoles. (**b**) Toluidine blue staining. (**c**) Immunostaining with CD79 antibody. Cytoplasmic signal is noted. Bars = 100μm. (**A17**)

The role of the Th2 T cells response in the control of GIN infections has been demonstrated in numerous studies using murine models of GIN infections in which specific cytokine manipulation is currently achieved (Patel et al., 2009). Consequently, in sheep the studies conducted in order to characterize the protective immune response have chosen to monitor the expression of candidate genes, known to be implicated in the protective immune response against extracellular pathogens in the murine model (Finkelman et al., 2004). It appears that the tendency was to attribute susceptibility or resistance in sheep to a CD4+ Th1 or Th2 immune response respectively. Indeed, an increased expression of genes involved in the Th2 immune response (i.e. IL-4, IL-5, IL-10, IL-13 and TNF-α) was reported in the intestinal mucosa (Ingham et al., 2008), the lymph draining the intestinal mucosa (Pernthaner et al., 2005), the lymph nodes (Andronicos et al., 2010) and the peripheral blood (Shakya et

al., 2009) of resistant compared with susceptible genotypes. These results indicate that in sheep the protective response against GIN associated with the genetic resistance would be at least a "Th2-like" response. However, in some of these studies the concomitant expression of Th1 and Th2 related genes were observed in the lymph draining the intestinal mucosa (Hein et al., 2004; Pernthaner et al., 2005). Interestingly, in a recent study it has been shown that the resistance to *T. circumcincta* in lambs carrying the *DRB1*1101* allele (associated with reduced FEC and worm burden) was influenced by an earlier interplay between Th1, Th2 and T regulatory (Treg) responses genes (Hassan et al., 2011). In keeping with these results, the IL-5 gene over-expression was shown to remain high in the resistant Black Belly lambs during a *H. contortus* infection, while it was down regulated earlier in INRA 401 susceptible lambs (Lacroux et al., 2006). Altogether, these results suggest that beyond the level of expression and the type of effector molecules of the genetic resistance, their kinetics of expression should also be analysed. The question of the regulation of the protective immune response of resistant and susceptible genotypes remains to be addressed. The control of the immune response in the gut is ensured by Foxp3+ (Forkhead box p3) Treg (regulatory) cells, a population of CD4+ T cells able to inhibit and the proliferation and effector function of other T cells. Their key role in the pathophysiology of intestinal bowel disease in human and numerous laboratory animal models is well described (Mayne and Williams, 2013). It has been suggested that *T. circumcincta* induces a Treg-like cells response in sheep since numerous Foxp3+ Treg cells were identified within the abomasal mucosa, as in murine models of GIN infection (McNeilly et al., 2009). It is likely that an appropriate/inappropriate immunoregulatory response would be involved in the expression of genetic resistance/susceptibility. Further longitudinal studies, comparing the mucosal and the peripheral immune response of resistant and susceptible genotypes would be of great interest to identify the key events that underline this protective response.

3. Conclusion

Despite numerous projects aimed at investigating the mechanisms involved in genetic resistance, a standardized pattern of biological parameters predictive of GIN resistance or susceptibility has not yet been identified. Indeed, most studies have been confronted to major constraints: i) a high inter-individual variability and, ii) the difficulty of monitoring kinetics of local cellular changes and genes expression patterns with time of infection. It is crucial to take into account the fact that the objective is to understand the complex cross-talk between two organisms: the host and the parasite. Thus, my hypothesis is that the dynamic of the host

responses is more pertinent than targeting single time point analysis during the course of the infection. The specificity of the targeted tissues should also be considered, as the objective will be at least to sample live animals in a breeding program. Today it seems that all the ingredients are available to conduct further experiments while comparing resistant and susceptible breeds and/or lines of goats and sheep using advanced high-throughput tools (i.e. transcriptomic, proteomic, metabolomic). The real added value will come from the data analysis. An integrative biology approach will probably help to open new avenues for the characterization of a biomarker profiles predictive of the genetic resistance. In human medicine since the 2000s the use of molecules or pattern of molecules, called biomarkers, has revolutionized patient cares and personalized medicine. Biomarkers are useful for accurate and rapid diagnostic of complex diseases (diagnostic biomarkers), to evaluate the development of the disease in an untreated individual (prognostic biomarkers) and to evaluate individually therapeutic response to a specific treatment (predictive biomarkers) (Oldenhuis et al., 2008; Ferlini et al., 2013). The identification of such molecules predictive of the resistance and/or susceptibility to GIN infection phenotypes could be of great interest. The evaluation of the genetic parameters (i.e. heritability and genetic correlation with the breeding objectives) would be a prerequisite for the validation of this trait in breeding programs (Lagarrigue and Tixier-Boichard, 2011). The key point that will allow the transition from the discovery to the innovation on the use of predictive biomarkers is the accessibility of the targeted tissue and the economic feasibility.

4. Perspectives

4.1. Identification of genes, genes networks and metabolites associated with the genetic resistance to GIN in Creole goats

Divergent selection of Creole goats based on EBV (Estimated Breeding Values) for FEC of does and sires was initiated in 2010 at the INRA PTEA facility. The divergence between the resistant and the susceptible lines was 1.1 genetic standard deviation between sires and 0.9 between does. In the frame of an European project, kids from these two divergent lines (n=192) have been genotyped with the 54k SNP chip to identify more precisely the QTL recently detected in Creole goats with the microsatellite technology (**A15**). The 192 kids from five successive cohorts were infected two times (challenges 1 and 2) with a single oral dose of 10,000 *H. contortus* third stage larvae. A number of "macro-phenotypes" were evaluated including the weekly monitoring of FEC, packed cell volume (PCV) and blood eosinophil counts. Additionally, the serum pepsinogen, the humoral response IgA and

IgE are currently under analysis. The genome-wide association study for detecting QTL affecting these traits is planned for 2015. These divergent lines are now the basis of the future work on the mechanisms underlying the genetic resistance.

My medium-term project aims at increasing the "phenotyping" deep to identify relevant genes and biomarkers patterns underlying the genetic resistance to GIN. My hypothesis is that it is probably more pertinent to lay stress on the dynamic of the host responses rather than to target on single time point analysis during the course of the infection. A first exploratory experiment has been conducted in 2014: Creole kids have been fistulated and biopsies sampling have been performed with a gastrofibroscop during the course of an experimental infection. The surgical procedure had been previously approved by the local ethic committee for animal experimentation. An experiment will be conducted at the end of 2015 with resistant and susceptible Creole kids (Figure 6).
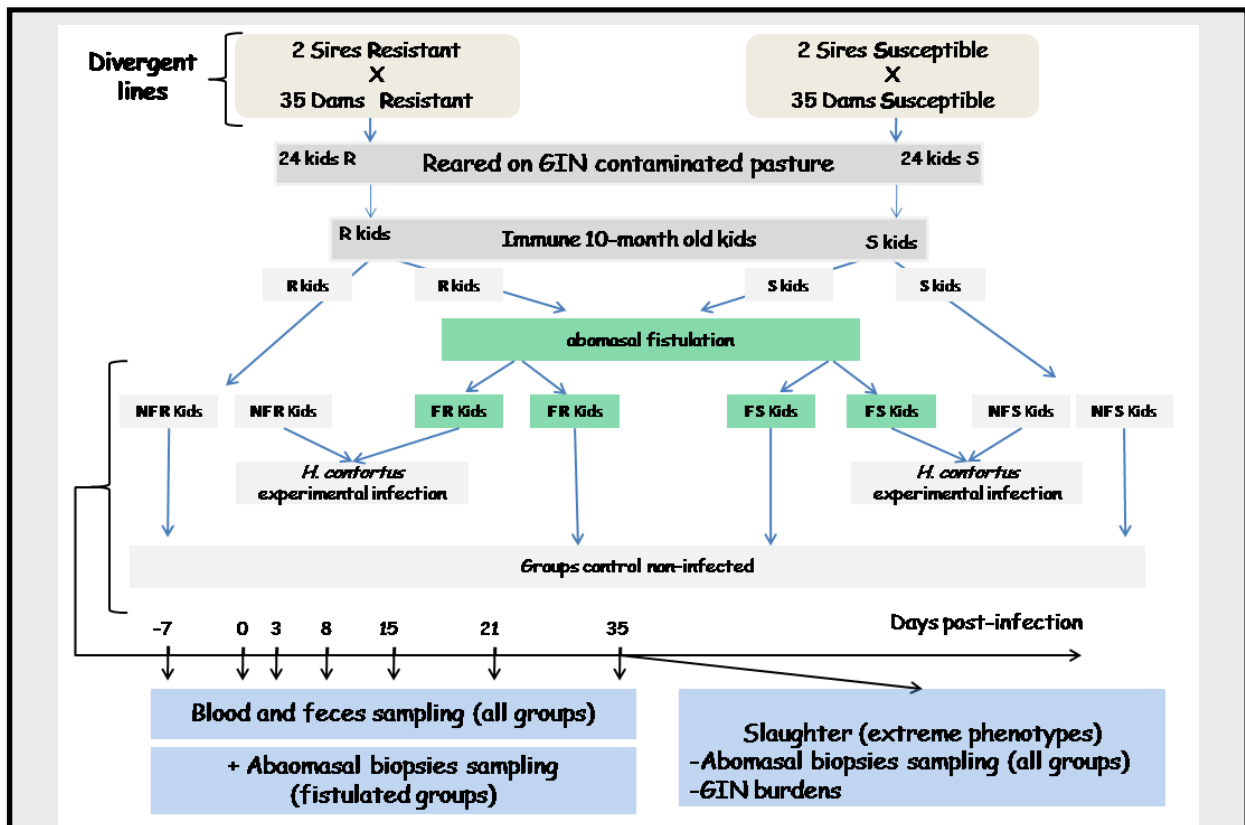
Figure 6. **Experimental design**. Immune kids (reared at pasture) will be separated into four groups: Abomasal Fistulated (**F**) Resistant (**R**) and Susceptible (**S**) kids and Non-Fistulated (**NF**) Resistant and Susceptible kids. In each group a subset of kids will be experimentally infected with *H. contortus* L3 and the others will not be infected (control non-infected). Samples will be taken from all kids during the course of the infection. At 35 days post-infection, kids chosen as extreme based on standard genetic deviation and also on FEC will be slaughtered for abomasal tissue sampling and GIN burdens quantification.

Transcriptomic analysis will be performed both on abomasal tissues and blood samples taken during the experiment. Histopathological changes in the abomasal mucosa will also be analyzed. At slaughter lymph node cell populations will also be sampled for transcriptomic analysis. In addition, blood metabolomic analysis will be performed in the course of the experiment. This project has been submitted as a *PhD* Thesis project in the frame of the European Graduate School in Animal Breeding in 2014, and selected for a 4 years funding. The work will start in September 2015 and will be jointly supervised by Elisabeth Jonas (Quantitative geneticist, Dept of Animal Breeding and Genetics, Uppsala University) and myself.

4.2. Selection for GIN resistance

The breeding program that will be develop by Nathalie Mandonnet (Quantitative geneticist at URZ) for Creole goat will offer a balanced selection which emphasizes long term profit with the improvement of adaptation traits (resistance and resilience). Moreover, this balanced breeding program will preserve a unique animal genetic resource through utilization. The breeding program will bring a high genetic progress. The parameters chosen (i.e. PCV and FEC) are synthetic criteria that should avoid co-evolution of resistance in worm burden. The fine analytic experimental approach described above should allow to identify at least the gene networks and metabolites involved in the genetic resistance or susceptibility to GIN infection. Beyond the interest of this work for the better understanding of the mechanisms underlying the genetic resistance to GIN in goats, one of the main objectives will be to identify the most pertinent biomarkers to implement this breeding scheme and thus increase genetic progress in adaptation of Creole goat to their harsh environment. Hence our objective is to investigate genetic variance available for selection for a pertinent biomarkers profile. Outputs from this project should thus provide new options for ecological intensification of small ruminant production, and produce a safer and higher quality product.

4.3. Immune function in Creole goats resistant to GIN

In the chapter II, we showed that it is likely that protective immune response against GIN associated with the genetic resistance is probably a Th2-like response. This response is specifically directed against extracellular pathogen. However, in the Caribbean, islands of the Indian Ocean and throughout sub-Saharan Africa, heartwater, a tick-born disease is also a major constraint on ruminant production (Allsopp, 2010). This disease induced by an obligate intracellular bacteria, *Ehrlichia ruminantium*, causes significant production losses particularly

in small ruminant, and threatens food security in endemic areas. The problematic is almost the same than that described for GIN, due to the emergence of acaricide-resistant ticks. To date only a blood-derived vaccine is commercially available and, its use limited to South Africa is not adapted to conditions prevailing in many parts of the world. There is still an effort of research for the development of an effective vaccine able to protect against homologous and heterologous strains at a low cost. There is a body of evidence suggesting that as an intracellular pathogen the protective response induced by *E. ruminantium* is a Th1 response (Winslow and Bitsaktsis, 2005). In 2010, a collaborative research program has been carried out in Guadeloupe between CIRAD CMAEE (Contrôle des Maladies Animales Exotiques et Emergentes) and INRA URZ to analyse the relationship between resistance to GIN and heartwater, both pathologies inducing two opposite immune responses. Eleven Creole bucks were evaluated on the resistance/susceptibility of their offsprings to an *E. ruminantium* standardized subletal infection inducing 70% of mortality (Vachiery et al., 2006). Two susceptible and 2 resistant bucks were chosen among them and were randomly mated to 22 does. Forty-three kids, allocated in 2 cohorts of 21 and 22 kids, were separated from mother and reared indoors in order to avoid transfer of mother immunity and tick infestation until the challenge at yearling. The intensity of the disease was quantified using clinical reaction indices (incubation period, intensity of fever, nervous signs, death) as already described by Vachiery et al. (2006). In this study, we highlighted for the first time genetic variability on resistance to heartwater (**C14**). The following step of this project will be to verify how this trait correlated to GIN resistance in Creole goats by comparing the divergent lines when the genetic standard deviation will have reached a least 2.

It should be noted that this experiment, consisting to induce a subletal infection at 70% of mortality, is difficult to implement essentially for ethic reason (the mortality rate). Thus an alternative would be to compare the immune function of PBMC (Peripheral Blood Mononuclear Cells) in response to specific antigens (*E. ruminantium* and *H. contortus*) and non-specific (concanavalin A, LPS) isolated from the divergent lines. This approach is largely used to characterize the immune status against a large variety of infections in humans (virus, bacteria or parasites) (Dong et al., 2012; Oliveira-Prado et al., 2012) and for a long time (David, 1973; Bloom, 1971; Herberman, 1978). The development of this project will be done in close collaboration with CIRAD CMAEE.

Chapter IV: Improve the host response through the nutritional way

1. Introduction

Initially, the question of the nutritional status has been addressed in my research work to avoid possible bias linked to this parameter on the expression of the resistance/susceptible genotypes. With time the approach has evolved towards the understanding of the trade-off between the major physiological functions, i.e the immune response against GIN, growth, reproduction and maintenance, in interaction with the host genotype. This part of my research work is more recent and thus less important in term of experiments comparatively to the work conducted on the characterisation of the mechanisms underlying the genetic resistance against GIN. Moreover, there is numerous recent literature reviews addressing the question of the nutritional manipulation of small ruminants to control GIN infection either in terms of practical tools to implement efficiently in husbandries (Torres-Acosta et al., 2012) or in terms of diet efficiently balanced in energy and protein (Houdijk, 2012). Thus, a succinct review of the literature will be presented here to contextualize the specific questions addressed in the experiments conducted in Creole goats and Black Belly sheep.

2. Interaction between the host nutrition and the response against GIN

2.1. Context

There is accumulating evidence showing that the nutritional status is closely associated with the capacity of the host to mount an efficient immune response against invading pathogens and more singularly against GIN (Adams, 2006; Colditz, 2008). Indeed, mounting an immune response is expensive both in terms of proteins and calories because of the metabolic requirement of immune cells, the synthesis of proteinaceous immune mediators and the repairing of damaged tissue (Lochmiller and Deerenberg, 2000). Minerals, trace elements and vitamins are also required for the development of immunity (Koski and Scott, 2003; McClure, 2003, 2008). Thus, nutritional manipulation of small ruminants has long been considered as a tool for the control of GIN infections (Clunies Ross, 1933; Gibson, 1963). It should be noted that in the literature the direct anthelmintic effects of plant secondary metabolites are also addressed as a mean to improve host nutrition (Athanasiadou et al.,
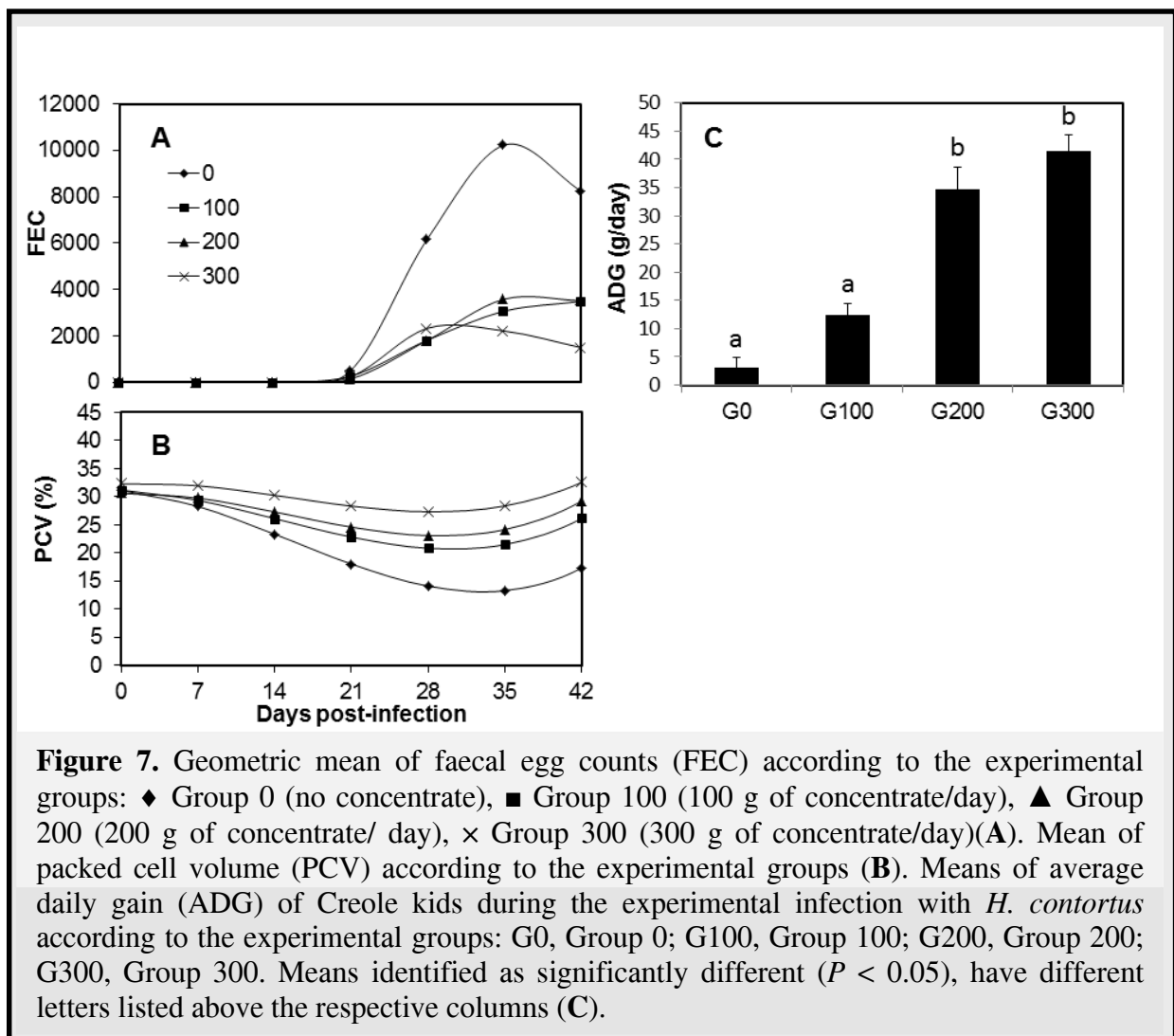
2008). These direct anthelmintic effects of plant secondary metabolites are not discussed here. Numerous feeding trials with small ruminants have paid much attention on the effects of an increased nutrients supply (in protein and/or calories) on the host response to GIN infections (Houdijk, 2012). It has been shown that an improved nutritional status could reduce the production losses and mortality rates due to GIN infection (Sykes and Coop, 2001; Walkden-Brown and Kahn, 2001). The respective roles of protein and energy supply to improve host resistance against GIN infections remains discussed. Moreover, the interactions between the genetic status (resistant vs. susceptible genotypes) and diet supplementation are less studied.

2.2. Effect of dietary supplementation on the resilience and resistance of Creole goats

The effect of supplementary feeding (balanced in energy and protein) on the response of Creole kids genetically resistant and susceptible to GIN infection, was evaluated during an experimental infection with *H. contortus* (**A12**). We showed that supplementary feeding in Creole kids was associated with increased resilience and resistance to *H. contortus* infection. This was shown by increased growth rate (Average Daily Gain, ADG), decreased excretion of GIN eggs in the faeces (FEC) and absence of acute anaemia in the supplemented groups compared to those not supplemented (Figure 7). Similar findings showing a significant effect of supplementation on resilience in browsing kids and in pen trials with goats have been reported (Blackburn et al., 1991; Torres-Acosta et al., 2004), as well as in field trials with grazing sheep (Vanhoutert et al., 1995). In sheep, numerous studies have suggested that the benefits of supplementation on the deleterious effects of GIN parasitism are more pronounced in susceptible genotypes compared to resistant ones (Coop and Kyriazakis, 1999). Similarly, we showed that susceptible kids were more responsive to the influence of increased supplementation, resulting in the absence of difference in resistance to infection between resistant and susceptible animals in the supplemented groups. In contrast, it has been reported that increased protein supplementation resulted in increased resistance to *H. contortus* infection in the native, more resistant Santa Ines lambs compared with the more susceptible Ile de France breed (Bricarello et al., 2005). Interestingly, this result has put in light the better capacity of the resistant genotype to survive in areas/breeding systems where forage quality is poor since the benefit of this more resistant genotype was not decreased by a lower protein diet.

Our results also suggested that the immune response against GIN infection of supplemented animals was enhanced compared to that of kids kept on a restricted diet and that eosinophils may play a role in this mechanism (**A12**). Indeed, supplementation enabled

blood eosinophilia to increase after infection whereas no variation was observed in non-supplemented groups. These results are in agreement with previous studies in Criollo kids from tropical Mexico under natural infection conditions and in sheep artificially infected with *H. contortus*, *T. colubriformis* or *T. circumcincta* (Vanhoutert et al., 1995; Datta et al., 1998; Valderrabano et al., 2002). In contrast, a significant higher level of the IgA response was found in non-supplemented animals compared to supplemented ones. These results are not consistent with a previous study in sheep which suggested that the plane of nutrition may be positively correlated with the antibody response against GIN (Martinez-Valladares et al., 2005). Nonetheless, these data confirmed that in our model of Creole goats the IgA response is better correlated with the nematode burden, as reflected by the FEC and the PCV.

Figure 7. Geometric mean of faecal egg counts (FEC) according to the experimental groups: ♦ Group 0 (no concentrate), ■ Group 100 (100 g of concentrate/day), ▲ Group 200 (200 g of concentrate/ day), × Group 300 (300 g of concentrate/day)(**A**). Mean of packed cell volume (PCV) according to the experimental groups (**B**). Means of average daily gain (ADG) of Creole kids during the experimental infection with *H. contortus* according to the experimental groups: G0, Group 0; G100, Group 100; G200, Group 200; G300, Group 300. Means identified as significantly different (*P* < 0.05), have different letters listed above the respective columns (**C**).

2.3. Trade-off between immunity against GIN infection and the other physiological functions and interaction with the host genotype

Numerous studies have shown that the response to an immunological challenge must be traded off against other physiological functions such as reproduction, growth and thermoregulation (Sheldon and Verhulst, 1996; Shudo and Iwasa, 2001; Zuk and Stoehr, 2002; van der Most et al., 2011).This trade-off between the major physiological functions, including the immune response against invading pathogens, is influenced not only by the host genotype and the physiological stage but also by environmental factors, particularly the availability and the quality of the feed in the ecosystem (Lochmiller and Deerenberg, 2000). By the use of a mathematical model, Vagenas and colleagues showed a higher significant effect of the nutritional status on GIN resistance traits in sheep than the effect of the host genotype, suggesting that discrepancies between published genetic parameters for GIN resistance may be function of environmental factors rather than differences in host genotype (Vagenas et al., 2007a; Vagenas et al., 2007b). We addressed this question experimentally, by evaluating the long-term effect of the nutritional history and its interaction with the host specie on the physiological trade-off between growth and immunity against GIN infection in two animal models differing in their growth potential and their level of GIN resistance (i.e. Black Belly sheep and Creole goats) (**B1**).

Lambs and kids were subjected to three distinct nutritional conditions at weaning: low, requirement and high dietary conditions. This 3-months period was followed by a 1-month period of nutritional requirement for all the animals before an experimental *H. contortus* infection. We showed an interaction between the host specie and the nutritional history for growth and the response against *H. contortus* (Figure 8). The response against *H. contortus*, monitored through the FEC, the blood eosinophil counts and the growth rate (ADG) were significantly affected by the nutritional history in lambs but not in kids. The lower FEC was found for lambs placed in high dietary conditions, however in the same time body weight loss (negative ADG) was observed in this group but not in kids with the same nutritional history. Among animals placed in low dietary conditions, kids were more resistant than lambs and the ADG was higher in lambs. Interestingly, a significant negative phenotypic correlation ($r = -0.49$, $P < 0.001$), was found in lambs between FEC and blood eosinophil counts but not for kids. This result supports the hypothesis of an increased immune response in lambs placed in high dietary conditions (blood eosinophil counts were considered as marker of the host response), suggesting a significant effect of the nutritional history on a potential trade-off for growth against the immune response in lambs.
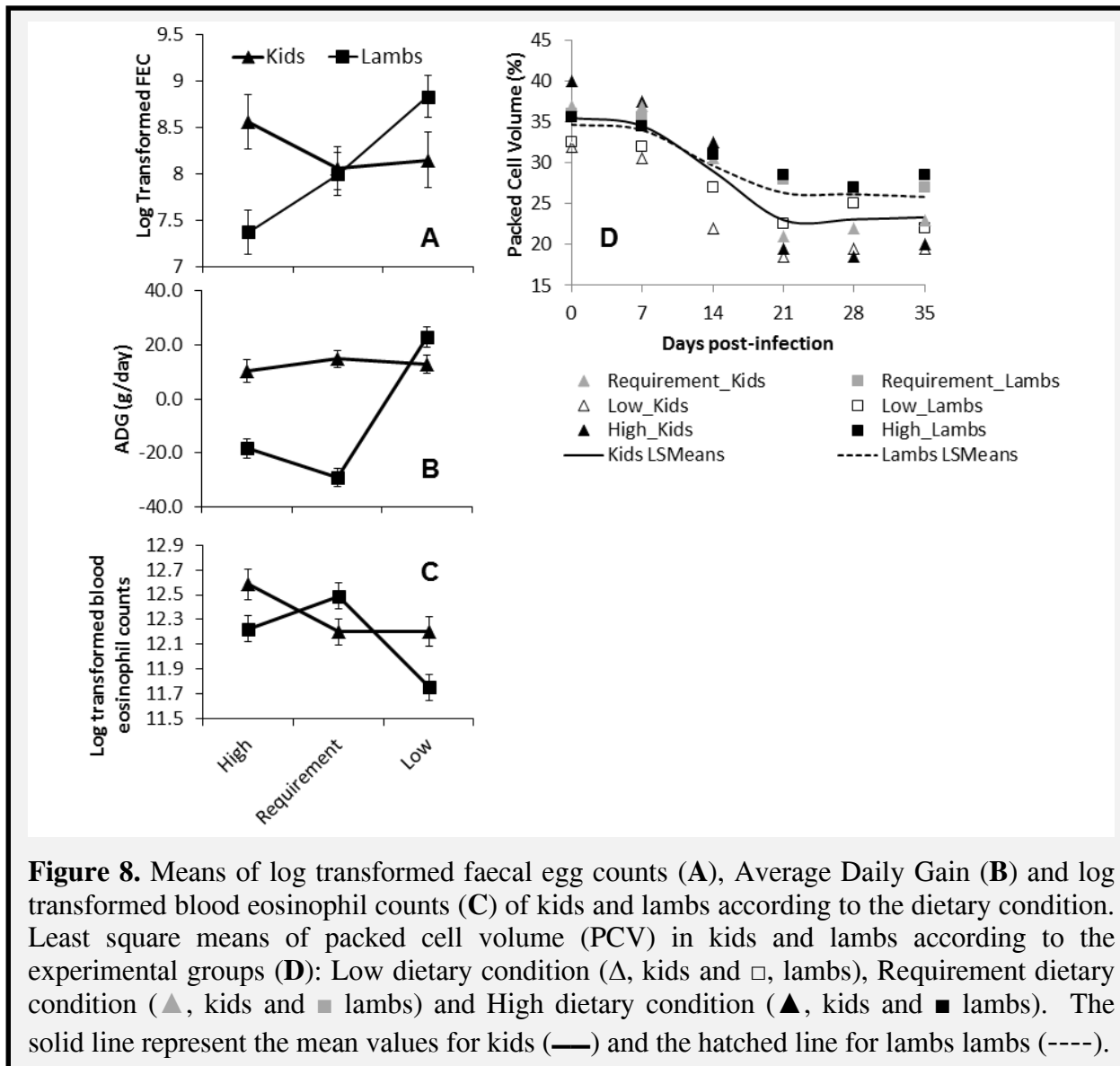
Figure 8. Means of log transformed faecal egg counts (**A**), Average Daily Gain (**B**) and log transformed blood eosinophil counts (**C**) of kids and lambs according to the dietary condition. Least square means of packed cell volume (PCV) in kids and lambs according to the experimental groups (**D**): Low dietary condition (∆, kids and □, lambs), Requirement dietary condition (▲, kids and ■ lambs) and High dietary condition (▲, kids and ■ lambs). The solid line represent the mean values for kids (——) and the hatched line for lambs lambs (----).

Similarly it has been shown that a short-term nutritional supplementation early after weaning potentiated a long-term resistance to GIN infection but in contrast with the present study no evidence for compensatory growth was observed for the animals placed in lower dietary conditions (Datta et al., 1999). It has been shown for a long time that in growing animals a period of nutritional restriction generates a compensatory growth. This compensatory growth phenomenon is the result of the optimization of different physiological functions of the animal whose main objective is to increase the growth rate (Hoch et al., 2003). In our trial, compensatory growth was observed in lambs with a lower response to *H. contortus* (higher FEC and lower eosinophilia). By using mathematical models, it has been shown that the host nutritional status could affect the interrelationship between host growth and resistance to pathogens (Vagenas et al., 2007b; Doeschl-Wilson et al., 2009). In contrast with our trial, in these models the infections were considered concomitantly with the host

nutritional status. Interestingly, here the nutritional history could also impact the response of the host to a nematode infection and this effect is host-dependent. Indeed, in kids no effect of the nutritional history was observed on the response against the nematode infection and the growth rate. In low dietary condition, the priority of the Black Belly lambs was the growth function at the expense of the response against a GIN infection, whereas in the high dietary condition the priority was the response against the GIN infection. In contrast, despite a severe pathophysiological impact, the Creole kids used the robustness strategy; their priority was the growth during the GIN infection whatever the nutritional history.

3. Conclusion

The benefit of diet supplementation on resistance and resilience of small ruminants to GIN infection has been reported in numerous studies. It appears that the limiting factor for the expression of host resistance is the availability in metabolizable protein in the diet rather than the metabolizable energy. It is hypothesized that proteins allow the expression of host resistance to GIN infection by fueling the increase in amino acid demand of the immune system. Consequently, beyond the quantity of protein in the diet to control GIN infection in small ruminants, the quality in terms of amino acid composition should be considered. Moreover it is very likely that the maintenance of an effective immune system and the development of a protective immune response are costly. Therefore, the potential trade-offs between production functions and resistance to GIN (and more largely the immune function) should also be studied. Indeed, selection of small ruminants for increased resistance to GIN should not compromise the productivity traits.

4. Perspectives
4.1. Impact of the nutritional status on nutrient partitioning during GIN infection in Small ruminants

We have seen that GIN infection of small ruminants resulted in decreased productivity arising from reducing feed intake, digestibility and repartitioning of nutrients to the gastrointestinal tract for the immune response and tissue repair (chapter II). Recently, is has been shown that divergent selection for resistant to *H. contortus* in sheep has been associated with specific partitioning of amino acid resources resulting in a larger nutritional cost compared with selection for decreased resistance (Doyle et al., 2014).

We have started a *PhD* project co-supervised by H. Archimède and myself at URZ in 2015 (Université des Antilles Doctorale School). The response (parasitological,

immunological, growth rate, intake and digestibility) of Black Belly lambs compared with Creole kids placed on four different nutritional status (from energy or protein supplementation to a balanced diet in energy and protein) following *H. contortus* infection will be analyzed during a 3-month period. Thus, the issue of amino acids partitioning (assessed with stable isotope labelled AA) will be addressed in interaction the host genotype (differing in the growth rate), the level of energy and the intensity of *H. contortus* infection. This project will be conducted in the frame of a collaboration with the team of I. Ortigues-Marty (Nutrients and Metabolism) from the INRA URH (Unité de Recherches sur les Herbivores, Theix) which has developed a recognized expertise in the study of nutrient partitioning in ruminants.

The same type of study will be engaged in order to evaluate if nutrient partitioning between the main physiological functions such as growth, reproduction, thermoregulation and the immune response to GIN would be involved in the physiological differences between divergent selection lines. Moreover, beyond the quantity of digestible protein available in the diet, this project could evolved toward the determination of amino acid profiles improving the host resistance to GIN. The multi-criteria (feed, environmental and health values) evaluation of unconventional plant resources for animal feeding is one important field of research for the agro-ecological development of animal production, basis of the URZ project. Thus, the ultimate objective of this work would be to provide qualitative criteria for the health value of the feed.

4.2. Molecular cross-talk between *H. contortus* and kids as affected with condensed Tannins

In this chapter the use of plant secondary metabolites as an alternative to synthetic anthelmintic has not been addressed. However, research works are conducted at the URZ on the anthelmintic effect of plant secondary metabolites (mainly condensed tannins, CT) both *in vitro* and *in vivo* (Marie-Magdeleine et al., 2009; 2010a; 2010b; 2010c). Indeed, during the last decades numerous bioactive CT-rich plants have been identified worldwide (Hoste et al., 2006). *In vivo* the anthelminthic effects were mainly based on faecal egg counts reduction (FECR), the most pertinent parameter to estimate nematode burden in live animals, worm burden (measured at slaughter) and improved animal performance (growth rate) both in livestock and laboratory animals (Butter et al., 2001; Hoste et al., 2006). Direct anthelmintic effects of purified CT have been shown in *in vitro* assays against numerous GIN species affecting livestock production (Hoste et al., 2012). Indeed, it has been demonstrated that CT could significantly inhibit or delay some key biological processes such as larval development

(egg development to infective larvae), motility and exsheathment. Few studies have investigated the effects of these CT treatments both *in vitro* and *in vivo* on further life traits of GIN within their host (e.g. effect on parasite's fitness or parasite's virulence) (Collas, 2015). Furthermore, the interaction between the levels of resistance/susceptibility of the host and the effect of CT treatment is never addressed in the studies. However, the characterisation of the mechanisms of action *in vivo* is essential in order to design efficient strategies of CT-rich plants utilization in GIN control. Moreover, according to Hoste et al (2006), an improved understanding of the underlying mechanisms would also provide precious knowledge about the risk of development of CT resistance in GIN. An exploratory project, aimed at addressing this issue has been constructed in collaboration with A. Blanchard-Letort (Molecular Parasitologist, UMR ISP, Infectiologie et Santé Publique, Tours) and Carine Marie-Magdeleine (Phytochemist, URZ). Considering the competence and skills of the group members, we will develop an original dynamic study of the molecular cross-talk between the host and the parasite over the time. We will take advantage of the experience on abomasal fistulation, to design an experimental approach allowing multiple sampling (i.e. animal biopsies and parasites) on live animals (fed with a resource rich in CT or not and infected with L3 larvae pre-treated *in vitro* with CT or not). For the first time, a kinetic of parasites' (transcriptomic profiles) and hosts' (immune response) modifications will be performed to better decipher the establishment and maintenance of the molecular cross-talk.

Chapter V: General conclusion

In this document I synthetized the research works conducted at URZ and PTEA, a tropical ecosystem rich in diversity and interaction. It would be commonplace to say that this work is the fruit of a team. No! It is more than that. At URZ and PTEA research is a team sport! What would be Zidane, Ronaldo or Messi without their team? Perhaps had they chosen tennis? More seriously, I have enjoyed during these years an environment rich in human quality and scientific skills within the URZ and PTEA also within other research units (cited in collaboration). In this favorable environment I develop an integrated research project from the characterization of the mechanisms underlying the genetic resistance to GIN to the interactions between the host genotype, the nutritional status and the response to GIN.

Thus today we have a standardized reproducible model of experimental infection of Creole goats with *H. contortus* which allows the expression of the genetic resistance (the dose of infective larvae, the animal nutritional status and the duration of the infection). The future experiments will be conducted with an original animal model, the divergent lines selected for increased resistance and susceptibility to GIN. More recently, we have developed a new experimental approach (abomasal canule), in order to have a dynamic vision of the cross-talk between the parasite and the host. Finally, we have developed "goat-specific" tools to study the immune response, e.g. ELISA (IgA, IgG and IgE), Real-Time PCR (IL-4, IL-5 and IL-13) and complete blood count. Altogether, these elements provide a solid foundation to progress in the knowledge of the genes networks and the mechanisms involved in the genetic resistance to GIN in goats and to identify potential biomarkers for GIN resistance. Our results could be of great interest for identification of pertinent biomarkers also in sheep and mostly for the selection in other breeds of goats. This painstaking work consisted in the development of the tools and the experimental model, gives a new breath to the studies of nutrition × parasitism interactions started at URZ in the 90's. In the coming years we address this work in terms of nutrients partitioning in interaction with the host genotype (divergent lines, temperate vs tropical breeds, selected vs non-selected breeds) and specie (sheep vs goats).

The perspectives described in this document will be conducted in the frame of the research project of URZ for 2013-2017 (*Promote in an Agro Ecological Perspective, efficient*

breeding systems in strong environmental constraints) and in close collaboration with researchers from URZ and other INRA and CIRAD units (Genphyse, URH, SELMET, CMAEE, ISP) and Uppsala University. The main objective will be expertise sharing with colleagues working with other animal species, other pathogens, in other disciplines (biostatistics, quantitative genetic, microbiology, nutrition, molecular parasitology) and in order to build collaborative research projects.

A thought for Steve Bishop an exceptional scientist gone too soon…

References cited

Abbott, E. M., J. J. Parkins, and P. H. Holmes. 1986. Vet. Parasitol. 20: 275-289.

Adams, C. A. 2006. Nutrition Research Reviews 19: 79-89.

Adams, D. B., and A. W. Cripps. 1977. Australian Journal of Experimental Biology and Medical Science 55: 509-522.

Adams, D. B., G. C. Merritt, and A. W. Cripps. 1980. Australian Journal of Experimental Biology and Medical Science 58: 167-177.

Alba-Hurtado, F., E. Romero-Escobedo, M. A. Munoz-Guzman, G. Torres-Hernandez, and C. M. Becerril-Perez. 2010. Vet. Parasitol. 172: 277-282.

Allsopp, B. A. 2010. Vet. Parasitol. 167: 123-135.

Amarante, A., P. Bricarello, J. Huntley, L. Mazzolin, and J. Gomes. 2005. Vet. Parasitol. 128: 99-107.

Amarante, A. F. T., P. A. Bricarello, R. A. Rocha, and S. M. Gennari. 2004. Vet. Parasitol. 120: 91-106.

Anderson, N., R. Blake, and D. A. Titchen. 1976. Parasitology 72: 1-12.

Anderson, N., J. Hansky, and D. A. Titchen. 1981. Parasitology 82: 401-410.

Anderson, N., J. Hansky, and D. A. Titchen. 1985. Int. J. Parasitol. 15: 159-165.

Anderson, R. C. (Editor), 2000. Nematode parasites of vertebrates: their development and transmission. 2nd Edition.

Andronicos, N., P. Hunt, and R. Windon. 2010. Int. J. Parasitol. 40: 417-429.

Athanasiadou, S., J. Houdijk, and I. Kyriazakis. 2008. Small Ruminant Research 76: 2-11.

Baker, R. L., and G. D. Gray. 2003. Australian Centre for International Agricultural Research (ACIAR), Monograph 113: pp. 63-95.

Bakert, R. L., J. O. Audho, E. O. Aduda, and W. Thorpe. 2001. Animal Science 73: 61-70.

Balic, A., V. M. Bowles, and E. N. T. Meeusen. 2002. Parasite Immunol. 24: 39-46.

Balic, A., C. P. Cunningham, and E. N. T. Meeusen. 2006. Parasite Immunol. 28: 107-115.

Barger, I. A. 1993. Int. J. Parasitol. 23: 463-469.

Beraldi, D., B. H. Craig, S. C. Bishop, J. Hopkins, and J. M. Pemberton. 2008. Int. J. Parasitol. 38: 1567-1577.

Beynon, S. A. 2012. Vet. Parasitol. 189: 113-124.

Bishop, S. C. 2012. Animal 6: 741-747.

Bishop, S. C., and C. A. Morris. 2007. Small Ruminant Research 70: 48-59.

Bishop, S. C., and M. J. Stear. 2003. Vet. Parasitol. 115: 147-166.

Blackburn, H. D. et al. 1991. Vet. Parasitol. 40: 99-112.

Bloom, B. 1971. New England Journal of Medecine 284: 1212–1213.

Bricarello, P. et al. 2005. Vet. Parasitol. 134: 99-109.

Bricarello, P. A. et al. 2002. Vet. Res. Commun. 26: 447-457.

Bricarello, P. A. et al. 2004. Small Ruminant Research 51: 75-83.

Bueno, L., A. Dakkak, and J. Fioramonti. 1982a. Parasitology 84: 367-374.

Bueno, L., C. Honde, G. Luffau, and J. Fioramonti. 1982b. Am. J. Vet. Res. 43: 1194-1199.

Butter, N. L., J. M. Dawson, D. Wakelin, and P. J. Buttery. 2001. J. Agric. Sci. 137: 461-469.

Cardia, D. F. F., R. A. Rocha-Oliveira, M. H. Tsunemi, and A. F. T. Amarante. 2011. Vet. Parasitol. 182: 248-258.

Charleypoulain, J., G. Luffau, and P. Pery. 1984. Vet. Parasitol. 14: 129-141.

Charlier, J., M. van der Voort, F. Kenyon, P. Skuce, and J. Vercruysse. 2014. Trends Parasitol. 30: 361-367.

Chartier, C. et al. 1998. Parasitol. Res. 84: 806-810.

Chiejina, S. N., and J. M. Behnke. 2011. Parasites & Vectors 4.

Clunies Ross, I., Gordon, H. McL. 1933. Aust. Vet. J. 9: 100-107.

Colditz, I. G. 2008. Parasite Immunol. 30: 63-70.

Collas, C., Salle, G., Dumont, B., Cabaret, J., Cortet, J., Martin-Rosset, W., Wimel, L., Fleurance, G. 2015. Journée de Recherche équine.

Connan, R. M. 1975. Parasitology 71: 239-246.

Coop, R. L., and I. Kyriazakis. 1999. Vet. Parasitol. 84: 187-204.

Coop, R. L., A. R. Sykes, and K. W. Angus. 1977. Res. Vet. Sci. 23: 76-83.

Coop, R. L., A. R. Sykes, and K. W. Angus. 1982. J. Agric. Sci. 98: 247-255.

Costa, C. A. F. et al. 2000. Vet. Parasitol. 88: 153-158.

Courtney, C. H., C. F. Parker, K. E. McClure, and R. P. Herd. 1984. Int. J. Parasitol. 14: 377-381.

Cripps, A. W., and J. W. Steel. 1978. Australian Journal of Experimental Biology and Medical Science 56: 181-194.

Cuquerella, M., M. T. Gomezmunoz, and J. M. Alunda. 1991. Vet. Parasitol. 38: 131-143.

Datta, F. U., J. V. Nolan, J. B. Rowe, and G. D. Gray. 1998. Int. J. Parasitol. 28: 1269-1278.

Datta, F. U., J. V. Nolan, J. B. Rowe, G. D. Gray, and B. J. Crook. 1999. Int. J. Parasitol. 29: 479-488.

David, J. 1973. New England Journal of Medecine: 143-149.

Davies, G., M. J. Stear, and S. C. Bishop. 2005. Animal Science 80: 143-150.

De la Chevrotiere, C., C. Moreno, P. Jaquiet, and N. Mandonnet. 2011. Inra Productions Animales 24: 221-233.

Delgado, C. L. 2003. J. Nutr. 133: 3907S-3910S.

Dhanda, J. S., D. G. Taylor, P. J. Murray, R. B. Pegg, and P. J. Shand. 2003. Asian-Australasian Journal of Animal Sciences 16: 1842-1852.

Diez-Tascon, C. et al. 2005. Physiol. Genomics 21: 59-69.

Doeschl-Wilson, A. B., W. Brindle, G. Emmans, and I. Kyriazakis. 2009. Plos One 4.

Dominik, S. 2005. Genet. Sel. Evol. 37: S83-S96.

Dong, H. L., I. Rowland, and P. Yaqoob. 2012. British Journal of Nutrition 108: 459-470.

Douch, P. G. C., R. S. Green, and P. L. Risdon. 1994. Int. J. Parasitol. 24: 921-928.

Doyle, E. K., L. P. Kahn, and S. J. McClure. 2014. Vet. Parasitol. 205: 175-185.

Doyle, E. K., L. P. Kahn, S. J. McClure, and J. M. Lea. 2011. Vet. Parasitol. 177: 316-323.

Fakae, B. B. et al. 2004. Vet. Parasitol. 122: 51-65.

Ferlini, A., C. Scotton, and G. Novelli. 2013. Public Health Genomics 16: 313-321.

Fernández, A. S. 1997. Proceedings of a workshop organized by FAO and the Danish Centre for Experimental Parasitology lpoh, Malaysia
 39-42.

Finkelman, F. D. et al. 2004. Immunol. Rev. 201: 139-155.

French, A. T. et al. 2008. Int. J. Parasitol. 38: 467-475.

Gamble, H. R., and A. M. Zajac. 1992. Vet. Parasitol. 41: 211-225.

Gibbs, H. C., and R. P. Herd. 1986. Veterinary Clinics of North America-Food Animal Practice 2: 211-224.

Gibson, T. E. 1963. The Proceedings of the Nutrition Society 22: 15-20.

Gill, H. S., G. D. Gray, D. L. Watson, and A. J. Husband. 1993a. Parasite Immunol. 15: 61-67.

Gill, H. S., D. L. Watson, and M. R. Brandon. 1993b. Immunology 78: 43-49.

Gray, G. D. 1997. Vet. Parasitol. 72: 345-366.

Greer, A. W., M. Stankiewicz, N. P. Jay, R. W. McAnulty, and A. R. Sykes. 2005. Animal Science 80: 89-99.

Gregg, P., J. K. Dineen, T. L. W. Rothwell, and J. D. Kelly. 1978. Vet. Parasitol. 4: 35-48.

Gregory, P. C. et al. 1985. Parasitology 91: 381-396.

Gruner, L. et al. 2004. Genet. Sel. Evol. 36: 217-242.

Gunia, M. et al. 2013. Animal 7: 22-33.

Gunia, M., F. Phocas, R. Arquet, G. Alexandre, and N. Mandonnet. 2011. J. Anim. Sci. 89: 3443-3451.

Harrison, G. B. L. et al. 2003a. Parasite Immunol. 25: 45-53.

Harrison, G. B. L., H. D. Pulford, W. R. Hein, W. B. Severn, and C. B. Shoemaker. 2003b. Parasite Immunol. 25: 79-86.

Hassan, M., J. P. Hanrahan, B. Good, G. Mulcahy, and T. Sweeney. 2011. Vet. Res. 42.

Hein, W., T. Barber, S. Cole, L. Morrison, and A. Pernthaner. 2004. J. Immunol. Methods 293: 153-168.

Henderson, N., and M. Stear. 2006. Vet. Immunol. Immunopathol. 112: 62-66.

Herberman, R. 1978. Investigative & Cell Pathology: 227-248.

Herrero, M., and P. K. Thornton. 2013. Proceedings of the National Academy of Sciences of the United States of America 110: 20878-20881.

Hoch, T., C. Begon, I. Cassar-Malek, B. Picard, and I. Savary-Auzeloux. 2003. Productions Animales 16: 49-59.

Honde, C., and L. Bueno. 1982a. Exp. Parasitol. 54: 371-378.

Honde, C., and L. Bueno. 1982b. Journal De Physiologie 78: A16-A16.

Hope, J. C., P. Sopp, S. Wattegedera, and G. Entrican. 2012. Small Ruminant Research 103: 23-27.

Hoste, H., C. Chartier, and Y. Le Frileux. 2002. Vet. Res. 33: 531-545.

Hoste, H., F. Jackson, S. Athanasiadou, S. M. Thamsborg, and S. O. Hoskin. 2006. Trends Parasitol. 22: 253-261.

Hoste, H., H. Leveque, and P. Dorchies. 2001. Vet. Parasitol. 101: 127-135.

Hoste, H. et al. 2012. Vet. Parasitol. 186: 18-27.

Hoste, H., J. F. Torres-Acosta, and A. J. Aguilar-Caballero. 2008. Parasite Immunol 30: 79-88.

Houdijk, J. G. M. 2012. Small Ruminant Research 103: 41-49.

Huntley, J. F., G. F. J. Newlands, F. Jackson, and H. R. P. Miller. 1992. Parasite Immunol. 14: 429-440.

Huntley, J. F. et al. 2001. Parasite Immunol. 23: 227-235.

Ingham, A., A. Reverter, R. Windon, P. Hunt, and M. Menzies. 2008. Int. J. Parasitol. 38: 431-442.

Jackson, F., and R. L. Coop. 2000. Parasitology 120: S95-S107.

Jones, W. O., and L. E. A. Symons. 1982. Int. J. Parasitol. 12: 295-301.

Keane, O. M. et al. 2006. BMC Genomics 7.

Kemp, J. M., N. A. Robinson, E. N. T. Meeusen, and D. M. Piedrafita. 2009. Int. J. Parasitol. 39: 1589-1594.

Koski, K. G., and M. E. Scott. 2003. J. Trace Elem. Exp. Med. 16: 237-251.

Kyriazakis, I. 2014. Animal Production Science 54: 1190-1197.

Kyriazakis, I., B. J. Tolkamp, and M. R. Hutchings. 1998. Anim. Behav. 56: 265-274.

Lacroux, C. et al. 2006. Vet. Res. 37: 607-622.

Lagarrigue, S., and M. Tixier-Boichard. 2011. Inra Productions Animales 24: 377-386.

Laurenson, Y. C. S. M., I. Kyriazakis, and S. C. Bishop. 2012. J. Anim. Sci. 90: 2167-2180.

Lawton, D. E. B., H. Wigger, D. C. Simcock, and H. V. Simpson. 2002. Vet. Parasitol. 104: 243-255.

Lindqvist, A., B. L. Ljungstrom, O. Nilsson, and P. J. Waller. 2001. Acta Veterinaria Scandinavica 42: 377-389.

Liu, S. M. et al. 2005. Livestock Production Science 97: 117-129.

Lochmiller, R. L., and C. Deerenberg. 2000. Oikos 88: 87-98.

MacKinnon, K. M., A. M. Zajac, F. N. J. Kooyman, and D. R. Notter. 2010. Parasite Immunol. 32: 484-493.

Mandonnet, N. et al. 2001. J. Anim. Sci. 79: 1706-1712.

Mandonnet, N. et al. 2005. Vet. Parasitol. 134: 249-259.

Mandonnet, N., V. Ducrocq, R. Arquet, and G. Aumont. 2003. J. Anim. Sci. 81: 2401-2408.

Mandonnet, N. et al. 2006. Animal Science 82: 283-287.

Marie-Magdeleine, C., H. Hoste, M. Mahieu, H. Varo, and H. Archimede. 2009. Vet. Parasitol. 161: 99-105.

Marie-Magdeleine, C., M. Mahieu, S. D'Alexis, L. Philibert, and H. Archimede. 2010a. Res. Vet. Sci. 89: 88-92.

Marie-Magdeleine, C., M. Mahieu, L. Philibert, P. Despois, and H. Archimede. 2010b. Small Ruminant Research 93: 10-18.

Marie-Magdeleine, C., L. Udino, L. Philibert, B. Bocage, and H. Archimede. 2010c. Vet. Parasitol. 173: 85-92.

Martinez-Valladares, M., M. P. Vara-Del Rio, M. A. Cruz-Rojo, and F. A. Rojo-Vazquez. 2005. Parasite Immunol. 27: 219-225.

Mayne, C. G., and C. B. Williams. 2013. Inflammatory Bowel Diseases 19: 1772-1788.

McClure, S. J. 2003. Australian Journal of Experimental Agriculture 43: 1455-1461.

McClure, S. J. 2008. Parasite Immunol. 30: 89-100.

McClure, S. J., D. L. Emery, B. M. Wagland, and W. O. Jones. 1992. Int. J. Parasitol. 22: 227-234.

McLeod, R. S. 1995. Int. J. Parasitol. 25: 1363-1367.

McNeilly, T. N., E. Devaney, and J. B. Matthews. 2009. Parasite Immunol. 31: 347-356.

Miller, H. R. P. 1986. The ruminant immune system in health and disease.: 496-524.

Miller, H. R. P. 1996. Vet. Immunol. Immunopathol. 54: 331-336.

Murphy, L. et al. 2010. Parasitology 137: 1249-1260.

Nicholls, C. D., P. R. Hayes, and D. L. Lee. 1987. Journal of Comparative Pathology 97: 299-308.

Nimbkar, C., P. M. Ghalsasi, A. A. Swan, S. W. Walkden-Brown, and L. P. Kahn. 2003. Animal Science 76: 503-515.

Norman, G. A. 1991. Developments in Meat Science 5: 57-87.

O'Connor, L. J., S. W. Walkden-Brown, and L. P. Kahn. 2006. Vet. Parasitol. 142: 1-15.

O'Sullivan, B. M., and A. D. Donald. 1973. Int. J. Parasitol. 3: 521-530.

Okon, E. D. 1980. Bulletin of animal health and production in Africa. Bulletin des sante et production animales en Afrique 28: 155-158.

Oldenhuis, C. N., S. F. Oosting, J. A. Gietema, and E. G. de Vries. 2008. Eur J Cancer 44: 946-953.

Oliveira-Prado, R. et al. 2012. BMC Infect. Dis. 12.

Papadopoulos, E. 2008. Small Ruminant Research 76: 99-103.

Parkins, J. J., P. H. Holmes, and K. C. Bremner. 1973. Res. Vet. Sci. 14: 21-28.

Patel, N., T. Kreider, J. F. Urban, and W. C. Gause. 2009. Int. J. Parasitol. 39: 13-21.

Perez, J. et al. 2001. Vet. Res. 32: 463-473.

Pernthaner, A., S. A. Cole, L. Morrison, and W. R. Hein. 2005. Infect. Immun. 73: 2175-2183.

Poppi, D. P., J. C. Macrae, A. Brewer, and R. L. Coop. 1986. British Journal of Nutrition 55: 593-602.

Presson, B. L., G. D. Gray, and S. K. Burgess. 1988. Parasite Immunol. 10: 675-680.

Rahman, W. A., and G. H. Collins. 1992. Vet. Parasitol. 43: 85-91.

Robinson, N., D. Piedrafita, K. Snibson, P. Harrison, and E. N. Meeusen. 2010. Vet. Res. 41.

Rose, H., T. Wang, J. van Dijk, and E. R. Morgan. 2015. Ecol. Model. 297: 232-245.

Roseby, F. B. 1977. Aust. J. Agric. Res. 28: 155-164.

Roseby, F. B., and R. A. Leng. 1974. Aust. J. Agric. Res. 25: 363-367.

Rout, P. K., K. K. Chauhan, O. Matika, and S. C. Bishop. 2011. Vet. Parasitol. 180: 315-322.

Rowe, A., C. Gondro, D. Emery, and N. Sangster. 2008. Vet. Parasitol. 154: 71-81.

Rowe, A., C. Gondro, D. Emery, and N. Sangster. 2009. Vet. Parasitol. 161: 76-87.

Rowe, J. B., J. V. Nolan, G. Dechaneet, E. Teleni, and P. H. Holmes. 1988. British Journal of Nutrition 59: 125-139.

Safari, E., N. M. Fogarty, and A. R. Gilmour. 2005. Livestock Production Science 92: 271-289.

Safari, E., N. M. Fogarty, and A. R. Gilmour. 2006. Australian Journal of Experimental Agriculture 46: 283-290.

Schallig, H. 2000. Parasitology 120: S63-S72.

Scott, I. et al. 1998. Int. J. Parasitol. 28: 1383-1392.

Scott, I. et al. 2000. Vet. Parasitol. 89: 79-94.

Shakya, K. P., J. E. Miller, and D. W. Horohov. 2009. Vet. Parasitol. 163: 57-66.

Sheldon, B. C., and S. Verhulst. 1996. Trends Ecol. Evol. 11: 317-321.

Shudo, E., and Y. Iwasa. 2001. J. Theor. Biol. 209: 233-247.

Simcock, D. C. et al. 1999. Int. J. Parasitol. 29: 1053-1063.

Simpson, H. V., D. E. B. Lawton, D. C. Simcock, G. W. Reynolds, and W. E. Pomroy. 1997. Int. J. Parasitol. 27: 825-831.

Simpson, H. V., B. H. Simpson, D. C. Simcock, G. W. Reynolds, and W. E. Pomroy. 1999. New Zealand Veterinary Journal 47: 20-24.

Spoor, M. S., A. B. Royal, and L. M. Berent. 2011. Veterinary Clinical Pathology 40: 136-136.

Stear, M. J. et al. 2004. Parasitology 129: 363-369.

Stear, M. J. et al. 1995. Parasite Immunol. 17: 643-652.

Stear, M. J., S. C. Bishop, N. G. Henderson, and I. Scott. 2003. Animal health research reviews / Conference of Research Workers in Animal Diseases 4: 45-52.

Stear, M. J., B. Boag, I. Cattadori, and L. Murphy. 2009. Parasite Immunol. 31: 274-282.

Stear, M. J., S. Strain, and S. C. Bishop. 1999. Vet. Immunol. Immunopathol. 72: 213-218.

Strain, S. A. J. et al. 2002. Parasitology 124: 545-552.

Strain, S. A. J., and M. J. Stear. 2001. Parasite Immunol. 23: 527-531.

Sutherland, I. A., J. Shaw, and R. J. Shaw. 2010. Vet. Parasitol. 171: 300-304.

Sykes, A. R., and R. L. Coop. 1976. J. Agric. Sci. 86: 507-515.

Sykes, A. R., and R. L. Coop. 2001. New Zealand Veterinary Journal 49: 222-226.

Sykes, A. R., R. L. Coop, and K. W. Angus. 1977. Journal of Comparative Pathology 87: 521-529.

Terefe, G. et al. 2007a. Vet. Res. 38: 647-654.

Terefe, G. et al. 2007b. Parasite Immunol. 29: 415-424.

Torres-Acosta, J. F. J., and H. Hoste. 2008. Small Ruminant Research 77: 159-173.

Torres-Acosta, J. F. J. et al. 2004. Vet. Parasitol. 124: 217-238.

Torres-Acosta, J. F. J. et al. 2012. Small Ruminant Research 103: 28-40.

Vachiery, N. et al. 2006. Vaccine 24: 4747-4756.

Vagenas, D., S. C. Bishop, and I. Kyriazakis. 2007a. Parasitology 134: 1263-1277.

Vagenas, D., A. Doeschl-Wilson, S. C. Bishop, and I. Kyriazakis. 2007b. Int. J. Parasitol. 37: 1617-1630.

Vagenas, D. et al. 2002. Animal Science 74: 199-208.

Valderrabano, J., R. Delfa, and J. Uriarte. 2002. Vet. Parasitol. 104: 327-338.

van der Most, P. J., B. de Jong, H. K. Parmentier, and S. Verhulst. 2011. Funct. Ecol. 25: 74-80.

van Dijk, J., G. P. David, G. Baird, and E. R. Morgan. 2008. Vet. Parasitol. 158: 73-84.

van Wyk, J. A., and G. F. Bath. 2002. Vet. Res. 33: 509-529.

Vanhoutert, M. F. J., I. A. Barger, and J. W. Steel. 1995. Vet. Parasitol. 60: 283-295.

Venturina, V. M., A. G. Gossner, and J. Hopkins. 2013. Vet. Res. Commun. 37: 171-181.

Walkden-Brown, S. W., and L. P. Kahn. 2001. Nutritional modulation of resistance and resilience to gastrointestinal nematode infection - A review. In: International Symposium on New Challenges for Animal Science in a New Century, Sendai, Japan. p 912-924.

Waller, P. J., and P. Chandrawathani. 2005. Tropical Biomedicine 22: 131-137.

Waller, P. J., L. Rudby-Martin, B. L. Ljungstrom, and A. Rydzik. 2004. Vet. Parasitol. 122: 207-220.

Waller, P. J., and R. J. Thomas. 1975. Parasitology 71: 285-291.

Watson, T. G., R. L. Baker, and T. G. Harvey. 1986. Proceedings of the New Zealand Society of Animal Production 46: 23-26.

Winslow, G. M., and C. Bitsaktsis. 2005. Curr. Opin. Infect. Dis. 18: 217-221.

Woolaston, R. R., and G. D. Gray. 1991. Wool Technology and Sheep Breeding 39: 84-87.

Zajac, A. M., S. Krakowka, R. P. Herd, and K. E. McClure. 1990. Vet. Parasitol. 36: 221-235.

Zuk, M., and A. M. Stoehr. 2002. Am. Nat. 160: S9-S22.

Appendix 1: Administrative activity and collaborations

1. Administrative activity

Since 2009, I am responsible for the organization and the animation of the 'lab'meetings' at URZ. These meetings are an opportunity for all the lab'members (lab and facility technicians, students, researchers) to exchange from a scientific and a technical point of view of our research themes and a way to deeply integrate new skills in the team. The programming is to divide sessions between different lines of research and levels of responsibility (researchers, post-docs, graduate students and technicians) to maintain a balance. This responsibility allows me to have a broad view of research at the URZ, as each talk is preceded by an informal meeting with the speaker. We have an average of 3 meetings / month. It should be noted that when we welcome researchers for *PhD* / HDR defenses, collaborations or workshops, they are invited to present their research topic or significant advances in their field. In 2015, I plan to create a 'journal club' in collaboration with the researchers of URZ. The specificity of this club will be its transdiciplinarity (from the molecule to the farming system: animal genetics, genomics, nutrition, physiology, immunology, phytochemestry and farming system). The idea is to contribute to the creation of a dynamic leading from the (factual) multidisciplinarity of the URZ to its transdisciplinarity.

Since June 2013, I am responsible for the BEA structure of PTEA (Animal Welfare in the experimental unit). The main role of this administrative structure established in accordance with the European Union legislation, is to monitor the experimental procedures underway within the experimental facility taking into account animal welfare. The BEA has also an advisory role to the technical and the scientific staffs.

I am regularly invited to be member of selection boards for the recruitment of technicians in molecular biology/biochemestry (since 2007) both for CIRAD and INRA laboratories (UMR CMAEE, UR Agro-Système Tropicaux, URZ). I participated in the establishment of technical and written tests. In 2012, I chaired a selection board for the recruitment of a chemical research technician in the unit INRA Agro-Système Tropicaux. This activity allows to take a step back from the role of different actors in laboratories and their career.

2. Scientific collaborations

I was invited to be part of two doctoral thesis committees supervised by N. Vachiery and T. Lefrancois (UMR INRA-CIRAD, CMAEE). Loic Emboulé defended his thesis in 2010

(topic: Transcriptome Analysis of virulent and attenuated *E. ruminantium* and application to the second generation vaccine development). Ludovic Pruneau defended his thesis in 2012 (topic: Study of the pathogenicity of virulent strains of *E.ruminantium* by transcriptomics). This activity provides a vision of the research conducted in this laboratory and gives a vision of the possible collaborations between their projects / expertise and the research conducted at the URZ. Thus, occasional collaborations have been implemented with this team on their area of expertise on small ruminant immunology (1 publication), molecular biology (1 publication) and heartwater (1 congress communication, 1 manuscript to submit). In the future, the perspectives developed in the chapter III (part 3.3) will be conducted in close collaboration with this team.

More generally, much of our research is conducted in collaboration with other INRA laboratory of the Animal Genetics Division (UMR GenPhyse, Toulouse), the Animal Physiology and Livestock Systems Division (UMR SELMET, Montpellier) and the Animal Health Division (UMR IHAP, Toulouse). These collaborations resulted in the writing of research papers and the development of research proposals. In 2012 and 2013, two research proposals designing in close collaboration with teams of the GenPhyse laboratory have been submitted to the ANR (French Agency for research funding). The proposals were selected on the complementary list but unfortunately not funding. In 2015, a new collaboration have started to perform molecular parasitological analysis in the frame of an experiment under course (UMR ISP, Tours). We took advantage of this collaboration to coordinate the writing of a proposal (Chapter IV, part 3.2.) that have been submitted to the INRA Metaprogramme GISA (Integrated Management of Animal Health). From 2010 to 2014, I was part of a European consortium in the frame of a 3.5 year FP7-funded collaborated project (European Union). The main focus of the 3SR project (Sustainable Solutions for Small Ruminants) was to mine genomic information of sheep and goats to deliver a step-change in the understanding of the genetic basis of three traits (Mastitis susceptibility, Nematode resistance and Ovulation rate) that have an important influence on sustainable production, health and welfare.

3. Students Mentoring and Teaching

On average I manage 2-3 students / year (1 undergraduate, 2 graduates). The students come from different backgrounds: Biology, Agricultural sciences, Veterinary sciences. Their scope of action can be limited either to measures on animals either laboratory tests or sometimes both. Since 2011, I ensure the co-supervision of Willy Ceï, *PhD* student working on nutrition × parasitism interactions (defense planned for the end of 2015). In February 2015,

Steve Ceriac have started a *PhD* on the same field with the specific issue of nutrient partitioning (co-supervision). In september 2015, Hadeer Abodashy will started a *PhD* in the frame of the Erasmus Mundus AGS-ABG, which aims to identify genes and genes networks associated with genetic resistance to GIN in Creole goats (co-supervision with E. Jonas Uppsala University).

Between 2007 and 2011, I provided lectures and tutorials (100 hours / year in average) in metabolic biological chemistry and physiology from the 1st to the 3rd year Bachelor of Biology, and in 2nd year of medical studies. Since 2012, the investment for the development of collaborations and designing of research proposals resulted in the reduction of my teaching activity (limited to Master students lectures, 40 hours / years).

Appendix 2 : List of publications

A. ISI indexed publications

1. Gobert, A. P., **Bambou, J.-C**., Werts, C., Balloy, V., Chignard, M., Moran, A. P., Ferrero, R. L. *Helicobacter pylori* HSP 60 mediates IL-6 production by macrophages via a TLR-2-, TLR-4- and MyD88-independent mechanism. *Journal of Biological Chemistry*. **2004**; 279(1):245-50.

2. Menard, S., Candalh, C., **Bambou, J.-C**., Terpend, K., Cerf-Bensussan, N., Heyman, M. Lactic acid bacteria secrete metabolite retaining anti-inflammatory properties after intestinal transport. *Gut*. **2004**; 53(6):821-8.

3. Bambou, J.-C., Giraud, A., Menard, S., Begue, B., Rakotobe, S., Heyman, M., Taddei, F., Cerf-Bensussan, N., Gaboriau-Routhiau, V. *In vitro* and *ex vivo* activation of the TLR5 signaling pathway in intestinal epithelial cells by a commensal *Escherichia coli* strain. *Journal of Biological Chemistry*. **2004**; 279 (41): 42984-92).

4. Begue, B., Wajant, H., **Bambou, J.-C**., Dubuquoy, L., Siegmund, D., Beaulieu, J.-F., Canioni, D., Berrebi, D., Brousse, N., Desreumaux, P., Schmitz, J., Cerf-Bensussan, N., Ruemmele F. M. Implication of TNF-related apoptosis-inducing ligand in inflammatory intestinal epithelial lesions. *Gastroenterology*. **2006**; 130(7):1962-74.

5. Begue B, Dumant C, **Bambou J.-C**., Beaulieu JF, Chamaillard M, Hugot JP, Goulet O, Schmitz J, Philpott DJ, Cerf-Bensussan N, Ruemmele FM. Microbial induction of CARD15 expression in intestinal epithelial cells via toll-like receptor 5 triggers an antibacterial response loop. *Journal of Cellular Physiology*. **2006**; 209(2):241-52.

6. **Bambou, J.-C.**, Giraud, A., Gaboriau, V., Taddei, F., Cerf-Bensussan, N. The intestinal flora: the scales without the sword? Journal de la société de Biologie. **2006**; 200 (2):113-20.

7. Giraud A., Arous S., De Paepe M., Gaboriau-Routhiau V., **Bambou J.-C.**, Rakotobe S., Lindner A.B., Taddei F., Cerf-Bensussan N. Dissecting the genetic components of adaptation of Escherichia coli to the mouse gut. *PLoS Genetics*. **2008**; 4(1):e2.

8. Ferrero R.L., Avé P., Ndiaye D., **Bambou J.-C.**, Huerre M.R., Philpott D.J., Mémet S. NF-kappaB activation during acute *Helicobacter pylori* infection in mice. *Infection and Immunity*. **2008**; 76(2):551-61.

9. **Bambou J.-C**, de la Chevrotière C, Varo H, Arquet R, Kooyman FN, Mandonnet N. Serum antibody responses in Creole kids experimentally infected with *Haemonchus contortus*. *Veterinary Parasitology*. **2008**; 158 (4) :311-8.

10. **Bambou J.-C**, González-García E, de la Chevrotière C, Arquet R, Mandonnet N. Peripheral immune response in resistant and susceptible Creole kids experimentally infected with *Haemonchus contortus*. *Small Ruminant Research*. **2009**; 82 (1):34-39.

11. Bambou J.-C, González-García E., Arquet R., Archimède H., Alexandre G., Mandonnet N. Intake and digestibility of naïve kids differing in genetic resistance and experimentally parasitized with *Haemonchus contortus* in two successive challenges. *Journal of Animal Science*. **2009**. 87 (7) 2367-2375.

12. Bambou J.-C, H. Archimède, R. Arquet, M. Mahieu, G. Alexandre, E. González-Garcia, N. Mandonnet. Effect of dietary supplementation on resistance to experimental infection with *H. contortus* in Creole kids. *Veterinary Parasitology*. **2011** ;178(3-4):279-85.

13. Bambou J.-C, Gourdine J.-L., Grondin, R., Vachiery, N., Renaudeau, D. Effect of heat challenge on peripheral blood mononuclear cells viability: comparison of a tropical and temperate pig breed. *Tropical animal health and production*. **2011**; 43(8):1535-41.

14. de la Chevrotière C, **Bambou J.-C**, Varo H, Jacquiet P, Mandonnet N. Genetic analysis of the potential role of IgA and IgE responses against *Haemonchus contortus* in resistance in Creole goats. *Veterinary Parasitology*, **2012**; 186(3-4):337-43.

15. de la Chevrotière C, Bishop SC, Arquet R, **Bambou J.-C**, Schibler L, Amigues Y, Moreno C, Mandonnet N. Detection of quantitative trait loci for resistance to gastrointestinal nematode infections in Creole goats. *Animal Genetics*. **2012**; 43(6):768-75.

16. **Bambou J.-C**, Cei W, Camous S, Archimède H, Decherf A, Philibert L, Barbier C, Mandonnet N, González-García E. Effects of single or trickle *Haemonchus contortus* experimental infection on digestibility and host responses of naïve Creole kids reared indoor.*Veterinary Parasitology*. **2013**; 191(3-4):284-92.

17. **Bambou J.-C**, Larcher T, Ceï W, Dumoulin PJ, Mandonnet N. Effect of experimental infection with *Haemonchus contortus* on parasitological and local cellular responses in resistant and susceptible young Creole goats. *Biomedical Research International*. **2013**:902759.

18. Cei, W, **Bambou, J.- C**, ; Silou, F, Mounoussamy, F, Alexandre, G. Growth and carcass traits of Creole kids experimentally infected by *Haemonchus contortus*: effects of sex, housing conditions and slaughter weights. *Livestock Research for Rural Development.* **2014**; 26 (11): article 199.

19. Ceï W., Mahieu M., Philibert L., Arquet R., Alexandre G.., Mandonnet N., **Bambou J.- C**. Impact of the post-weaning parasitism level on genetic evaluation of resistance against gastrointestinal nematode infection in Creole kids. *Veterinary Parasitology*. **2015**; 207 (1-2): 166-169.

B. Manuscript submitted and in preparation

1. Ceï, W., Salah, N., Paut, C., Dumoulin, P.-J., Arquet, R., Félicité, Y., Alexandre, G., Archimède, H., **Bambou, J.-C**. Potential trade-off between growth and the response against *Haemonchus contortus* in small ruminants: effect of the nutritional history, host specie and their interaction. Submitted to Animal.

2. Bambou, J.-C., Mahieu, M., Gunia, M., Alexandre, G., Mandonnet, N. Genetic Resistance to Parasites in Small Ruminants: from Knowledge to Implementation in the Tropics. In prep for submission to Animal.

3. Cei, W., **Bambou, J.-C**., Alexandre, G., Archimede, H. Interaction between nutrition and parasitism in small ruminant: A meta-analysis. In prep for submission to Journal of Animal Science.

C. Conference presentations

1. Bambou, J.C., Mahieu, M., Arquet, R., Naves, M., Abinne-Molza, L., Varo, H., Mandonnet, N., (2006). Characterization of blood immunoglobulin responses to *Haemonchus*

contortus in resistant and susceptible Creole kids naturally infected with gastrointestinal strongyles. 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte (Brasil), 13-18 August 2006, p. 167.

2. Bambou, J.C., Gonzalez Garcia, E., de la Chevrotière, C., Arquet, R., Vachiéry, N., Mandonnet, M., (2008a). Experimental heamonchosis in resistant and susceptible Creole kids. Joint ADSA-ASAS Annual Meeting, Indianapolis, Indiana, USA, July 7-11, 2008a, p. 469.

3. Mandonnet, N., **Bambou, J.-C.**, Chevrotiere, C., Naves, M., Alexandre, G., Arquet, R., (2008b). Différents angles d'étude de la résistance génétique aux strongles chez les caprins créoles de Guadeloupe. Journées Scientifiques, Département de Génétique Animale, Lacanau, France, 21-24/04/2008, p. 56.

4. Bambou, J.C., De la Chevrotière, C., Arquet, R., Gonzalez Garcia, E., Mahieu, M., Archimède, H., Alexandre, G., Mandonnet, M.,(2008c). Genetic evaluation of resistance to strongyles in Creoles kids is affected by protein supplementation. 9th International Conference on Goats, Querataro MEXICO, 31 aout - 4 septembre 2008, p. 469.

5. González-García, E., **Bambou, J.C.**, Arquet, R., Mandonnet, N., (2008d). Haemonchus contortus infection affects feed intake and diet digestibility in Creole goats. 9th International Conference on Goats, Querétaro MEXICO, 31aout - 4 septembre 2008d, p. 489.

6. de la Chevrotière, C., Moreno, C., Arquet, R., **Bambou, J.C.**, Shibler, L., Amigues, Y., Alexandre, G., Mandonnet, M., (2008[e]). Preliminary detection of QTL associated with resistance to gastrointestinal nematode in the Creole Goat. In: 9th International Conference on Goats, Querataro MEXICO, 31 aout - 4 septembre 2008, p. 273.

7. de la Chevrotière, C., Bishop, S., Moreno, C., Arquet, R., **Bambou, J.C.**, Schibler, L., Amigues, Y., Mandonnet, N., (2009a). Identification of QTL associated with gastrointestinal nematode resistance in Creole goat. EAAP 60th annual congress, Barcelona, Sapin, 24-27 August 2009.

8. de la Chevrotière, C., **Bambou, J.C.**, Arquet, R., Jaquot, M., Mandonnet, N., (2009b). La sélection génétique pour la maîtrise des strongyloses : cas particulier de la chèvre Créole de Guadeloupe. 16ème Rencontres Recherches Ruminants, Paris (France), 2 et 3 décembre 2009, pp. 269-272.

9. Mahieu, M., Arquet, R., Fleury, J., Coppry, O., Marie-MagdeleineC., Boval, M., Archimède, H., Alexandre, G., **Bambou, J.-C.**, Mandonnet, N., (2009c). Contrôle intégré du parasitisme gastro-intestinal des petits ruminants en zone tropicale humide. 16ème Rencontres Recherches Ruminants, Paris (France), 2 et 3 décembre 2009, pp. 265-268.

10. Bambou, J.-C., Arquet, R., Mahieu, M., Mandonnet, N., (2010a). Impact of the type of experimental infection with Haemonchus contortus and post-weaning parasitism level on genetic evaluation of the resistance of Creole kids - Proceedings of the SAPT2010 conference. Advances in Animal Biosciences, pp. 407-408.

11. Bambou, J.-C., Cei, W., Barbier, C., Mandonnet, N., Gonzalez-Garcia, E., (2010b). Comparing the effects of single or trickle experimental infections with Haemonchus contortus

on digestibility and host response in naïve Creole kids reared indoors - Proceedings of the SAPT2010 conference. Advances in Animal Biosciences, pp. 410-410.

12. Bambou, J.-C., Grondin, R., Gourdine, J.-L., Renaudeau, D., (2010c). Effect of heat challenge on peripheral blood mononuclear cell viability: comparison of a tropical and a temperate pig breed - Proceedings of the SAPT2010 conference. Advances in Animal Biosciences, pp. 420-420.

13. Bambou, J.C., de la Chevrotière, C., Gunia, M., Arquet, R., Mandonnet, N., (2010d). Genetic correlation between resistance to strongyle natural infection and resistance to Haemonchus contortus experimental infection in Creole goats. Xth International Goat Congress, Recife, Brasil.

14. Bambou, J.C., Vachiery, N., Despois, P., Giraud-Girard, K., Arquet, R., Pinarello, V., Aprelon, R., Barbier, C., Gobardham, J., Mandonnet, N., Lefrançois, T., (2010e). Assessment of genetic variability of resistance to heartwater in Creole goats - Proceedings of the SAPT2010 conference. Advances in Animal Biosciences, pp. 408-409.

15. Cei, W., **Bambou, J.-C.**, Mahieu, M., Alexandre, G., (2010f). Carcass traits of male and female Creole goats according to slaughter weights, preliminary results - Proceedings of the SAPT2010 conference. Advances in Animal Biosciences, pp. 396-397.

16. de la Chevrotière, C., Gunia, M., Menendez-Buxadera, A., **Bambou, J.C.**, Mandonnet, N., (2010g). Genetic parameters of a gastrointestinal resistant trait in Creole goats during post-weaning period using a random regression model. 9th WCGALP Leipzig, Germany.

17. Gunia, M., Phocas, F., de la Chevrotière, C., **Bambou, J.-C**., Mandonnet, N., (2010h). Genetic parameters of resistance and growth traits for a breeding programme in Creole goats. 9th WCGALP, Leipzig, Germany.

18. Mahieu, M., Arquet, R., Alexandre, G., Boval, M., **Bambou, J.-C.**, Archimede, H., Marie-Magdeleine, C., Mandonnet, N., (2012a). Integrated control of goat gastrointestinal parasitism: an example in the humid tropics. XI International Conference on Goats (ICG 2012), Gran Canarias, Spain 24-27 September 2012.

19. Cei, W., **Bambou, J.C.**, Mandonnet, N., Alexandre, G., (2012b). Carcass characteristics of Creole goats genetically indexed for faecal egg count. Proc XI International Conference on Goats, Gran Canaria, Spain, 23-27 September 2012, pp. M-32, 238.

20. Bambou, J.C., Larcher, T., Ceï, W., P.-J., D., Mandonnet, N.,(2013a). Effect of *H. contortus* infection on parasitological and local cellular responses of Creole kids. 64th Annual Meeting of the EAAP, Nantes, France, 26-30 august 2013.

21. Ceï, W., Mahieu, M., Archimède, H., **Bambou, J.-C.**, Hiol, A., Alexandre, G. (2013b). Effect of experimental infection and diet supplementation on meat Creole goat performances. 64th Annual Meeting of the EAAP, Nantes, France, 26-30 august 2013.

22. **Bambou, J.C.**, Ceï, W., Dumoulin, P.-J., Mahieu, M., Mandonnet, N. Genetic evaluation of gastrointestinal nematode resistance in goats: Impact of the post-weaning parasitism level.

89th Annual Meeting of the American Society of Parasitologists. New-Orleans, United States, 2014.

23. **Bambou, J.C.**, Ceï, W., Dumoulin, P.-J., Alexandre, G., Archimède, H. Effect of energy and protein supplementation in lambs and kids on the response to *Haemonchus contortus*. 89th Annual Meeting of the American Society of Parasitologists. New-Orleans, United States, 2014.

D. Report diploma

1. Bambou, J.-C. (2001). Etude de l'activation de NF-κB par les bactéries du genre *Helicobacter* et son rôle dans l'inflammation gastrique. Master of Science. Université Denis Diderot (Paris VII).

2. Bambou, J.-C. (2004). Etude des interactions entre les cellules épithéliales intestinales et la flore commensale. *PhD* Thesis in Physiology et Pathophysiology. Université Pierre et Marie Curie (Paris VI).

E. Student reports

BSc Students

1.Dampied, J. (2007). Cinétique d'excrétion des œufs de *Haemonchus contortus* : étude comparative entre ovins et caprins. Rapport de stage Licence (Université de Cergy-Pontoise)

2.Claude, E. (2008). Etude de la réponse immunitaire humorale des cabris Créoles contre les strongles gastro-intestinaux. Rapport de stage de Licence (Université des Antilles et de la Guyane).

3.Lunion, S. (2008). Rôle de la réponse immune dans les infestations parasitaires chez les caprins Créole. Rapport de stage de Licence (Université des Antilles et de la Guyane).

4.Rambinaising, S. (2008). Bilan parasitaire de chevreaux infestés expérimentalement par *Haemonchus contortus*. Rapport de stage de Licence (Université des Antilles et de la Guyane).

5.Gonthier, D. (2009). Suivi d'une infestation expérimentale de cabris créoles par *Haemonchus contortus*. In Rapport BTS Production Animale (Tours)

6.Cely, T. (2009). Conséquences zootechniques des infestations par les nématodes gastro-intestinaux. Rapport BTS Production Animale (Guadeloupe).

7.Studer, A. 2012. Suivi d'une infestion expérimentale par *Haemonchus contortus* de deux lignées divergentes résistantes et sensibles de caprins Créole In BTSA Productions Animales (Vendôme).

8.Anger, F. 2013. Etude de la pertinence de l'OPG par rapport à la mesure d'excrétion totale pour évaluer le niveau d'infestation par les nematodes gastro-intestinaux chez les petits ruminants. BTSA Productions Animales (Vendôme).

9.Baltyde, K.-C. (2010). Infestation du parasite *Haemonchus contortus* et réponse immunitaire des caprins Créoles. Rapport de stage de Licence (Université des Antilles et de la Guyane).

10.Barthel, T. (2012). Suivie physiopathologique d une infestation experimentale par Haemonchus contortus. Rapport de stage de Licence (Université des Antilles et de la Guyane).

11.Polomack, V. (2012). Effets de l'infestation par *Haemonchus contortus* sur l'alimentation et la nutrition des caprins Créole. Rapport de stage de Licence (Université des Antilles et de la Guyane).

Msc Students

12.Bissonier, C. (2005). Réponse en immunoglobulines sériques de la chèvre Créole infestée par Haemonchus contortus : Mise au point du dosage et validation chez des chevreaux de statut résistant ou sensible. Master 1 Sciences de la Vie et de la Santé, (Université d'Auvergne Clermont 1).

13. Louis, J. (2007). Etude de la réponse immunitaire protectrice contre *Haemoncus contortus* chez les chevrettes Créole de statuts génétiques résistants ou sensibles. Master 1 Biologie cellulaire, physiologie, pathologies (Université Paris VII)

14.de la chevrotiere, C. (2007).Caractérisation de marqueurs génétiques de résistance aux strongles gastro-intestinaux chez les caprins Créole. Equivalent Master 2 Ecologie. (Faculté des Sciences, Université Sherbrooke, Québec, Canada). *Co-encadrement (50%)*.

15.Zeiger-Poli, C. 2011. Rôle des IgA sériques dans la résistance génétique des caprins Créole aux nématodes gastro-intestinaux. Msc Molecular genetics and Immunology. Edinburgh Napier University.

16.Silou, S. (2013). Mise au point du dosage du pepsinogène sérique chez les caprins. Master 1 de Biologie-Santé spécialité Génétique moléculaire et cellulaire. (Université de Bordeaux Segalen).

17.Bederina, M. (2013). Evaluation de la pertinence de nouveaux indicateurs de la résistance aux nématodes gastro-intestinaux. Master 2 Ecologie-biodiversité. Université de Montpellier 2.

18.Paut, C. (2013). Effets de l'alimentation et du parasitisme sur les performances des petits ruminants en croissance. Master 2 Ecologie-biodiversité Specialité maladies transmissibles : environnement, dynamique. Université de Montpellier 2.

19. Severin. A. Identification de biomarqueurs de la résistance génétique aux nématodes gastro-intestinaux. Master 2. Biologie et Santé. Université des Antilles et de la Guyane.

Agricultural and Veterinary Schools

20.Rouquet, P. (2006) - Rôle des immunoglobulines locales et de l'éosinophilie circulante dans la réponse protectrice des caprins Créole vis-à-vis des strongles. 2ème année vétérinaire. Ecole Nationale Vétérinaire de Toulouse.

21.Dumas, E. (2007). Etude de l'interaction entre complémentation et niveau d'infestation chez les chevrettes Créole. 5ème année vétérinaire. Certificat d'Etudes Approfondies Vétérinaires. Ecole Nationale Vétérinaire de Toulouse.

22.Guidez, N. (2007). Rôle de l'alimentation dans le niveau d'infestation des caprins Créole. 2ème année vétérinaire. Ecole Nationale Vétérinaire de Toulouse.

23.Decherf, A. (2009). Comparaison de deux modes d'infestations expérimentales par *Haemonchus contortus.*4ème année école ingénieur agronome. Institut Supérieur d'Agriculture de Lille.

24.Privat, S. (2010). Bilan parasitaire et sérologie de caprins infestés expérimentalement par *Haemonchus contortus*. 3ème année vétérinaire. Ecole Nationale Vétérinaire de Toulouse.

25.Bouharaoua, N., (2012). Etude des mécanismes physiologiques associés à la résistance des chèvres Créole aux strongles gastro-intestinaux. Thèse vétérinaire. Ecole Nationale Vétérinaire de Nantes (Oniris).

26. NKamba, I. Dosage des immunoglobulines A et du pepsinogènes sériques chez des animaux canulés et infestés par *Haemonchus contortus*. 2$^{\text{ème}}$ année école ingénieur agronome. AgroParistech.

Appendix 3 : Curriculum Vitae

Jean-Christophe BAMBOU
36 ans, marié, 3 enfants
1108F Hauteurs Lézarde
97170 Petit-Bourg
Portable : 06 90 31 72 74
Tél domicile   : 05 90 99 86 06

<div align="center">

<u>Formation Universitaire</u>

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nov. 2004	**Doctorat de Physiologie et Physiopathologie** Université de Paris VI Pierre et Marie Curie
2000-2001	**DEA Biologie et Pathologie des Epithéliums** Université Paris VII Denis Diderot
1999 – 2000	**Maîtrise Biologie Cellulaire et Physiologie** *mention immunologie et microbiologie médicale* Université Paris VI Pierre et Marie Curie

<div align="center">

<u>Expérience professionnelle en Enseignement et Recherche</u>

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novembre 2011	Recrutement en tant qu'ingénieur de recherche, INRA Unité de Recherches Zootechniques
sept 2010 à octobre 2011	Ingénieur de recherche contractuel, INRA Unité de Recherches Zootechniques
sept 2009 à août 2010	Attaché temporaire d'Enseignement et de Recherche, Université des Antilles et de la Guyane
mars 2005 à août 2009	Ingénieur de recherche contractuel, INRA Unité de Recherches Zootechniques
depuis mars 2007	Enseignant vacataire en Biochimie et Biologie Moléculaire, Université des Antilles et de la Guyane
sept. 2001 à nov. 2004	Thèse de Doctorat, INSERM U793 Hôpital Necker enfants malades (Paris)

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<u>Activités d'encadrement</u>

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Depuis mars 2005	du BTS au Master 2 et Ecoles vétérinaire : 26 étudiants encadrés et co-encadrés Doctorat : 2 étudiants co-encadrés à 50% (Thèse en cours : W. Ceï et S. Ceriac) Comité de thèse : 3 étudiants (C. de la Chevrotière, L. Emboulé et L. Pruneau : thèses soutenues en 2011 et 2012)

Expertise scientifique

Reviewer pour des revues internationales à comité de lecture : Parasites and vectors, Research in veterinary science, Tropical animal health and production, Animal (5 à 6 manuscrits/an) avec un IF moyen de 2.2

Financements

-Bourse départementale de post-doctorat (2005-2006)
-Participation au Contrat Plan Etat Région 2007-2013 (Agro-écologie de systèmes multi-espèces pour le développement d'une agriculture durable en milieu tropical) directement gérés par l'URZ
-Bourse régionale de post-doctorat 2010-2011
-Participation à un projet européen du 7ème PCRD (2011-2014): Sustainable Solution for Small Ruminants, participation WP3 sur la caractérisation des mécanismes sous-jacents la résistance génétique des petits ruminants aux NGI.
-Coordinateur du projet GoatOmics financé par le département de Génétique Animale de l'INRA
-Coordinateur d'un projet ANR Jeunes chercheurs(ses) (2012/2013) : liste complémentaire
-Responsable d'une tâche dans le projet Sustainable Treatment Reduction against Parasitism in livestock (2013-2017)
-Coordinateur d'un projet ANR Jeunes chercheurs(ses) (2013/2014) : pré-proposition retenue, projet final non-retenu
-Responsable d'une tâche dans le projet Maladies Infectieuses (CPER-PO 2014-2020)
-Coordinateur d'un projet exploratoire Métaprogramme GISA (2015) : avis d'éligibilité reçu en avril 2015, évaluation finale juillet 2015